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PART III

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Food and Drug Administration

■

DRUGS FOR HUMAN USE

Reorganization and Recodification

Title 21—Food and Drugs

CHAPTER I—FOOD AND DRUG ADMINISTRATION, DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

[Recodification Docket No. 6]

SUBCHAPTER D—DRUGS FOR HUMAN USE

Reorganization and Republication

The Commissioner of Food and Drugs, for the purposes of establishing an orderly development of informative regulations for the Food and Drug Administration, furnishing ample room for expansion of such regulations in years ahead, and providing the public and affected industries with regulations that are easy to find, read, and understand, has initiated a recodification program for Chapter I of Title 21 of the Code of Federal Regulations.

This is the sixth document in a series of recodification documents that will eventually include all regulations administered by the Food and Drug Administration.

The volume of regulatory material for human drugs under former Subchapter C—Drugs has necessitated recodifying these regulations in two documents. The fifth document in the series, which appeared in the FEDERAL REGISTER of March 29, 1974 (39 FR 11679), recodified in a new Subchapter D—Drugs for Human Use, certain human drug regulations, excluding antibiotic drugs for human use. This, the sixth document, recodifies, under the new Subchapter D, the antibiotic drugs for human use formerly under Parts 141 through 151j of Subchapter C.

The procedural regulations have been reorganized under Parts 430 through 436 of Subchapter D—Drugs for Human Use. The individual drug monographs for antibiotic drugs for human use, recodified under Parts 440 through 460, are organized so that individual antibiotic drugs are grouped together as a major class under each Part, based on structure and related activities or on special usage.

The Parts are structured to contain Subparts A through J, indicating a specific dosage form for each subpart as follows:

Subparts
A—Bulk Drugs
B—Oral Dosage Forms
C—Injectable Dosage Forms
D—Ophthalmic Dosage Forms
E—Otic Dosage Forms
F—Dermatologic Dosage Forms
G—Vaginal Dosage Forms
H—Rectal Dosage Forms
I—[Reserved]
J—Certain Other Dosage Forms

Each drug is keyed to a bulk drug section number under Subpart A which is carried throughout the various dosage forms. Each subpart contains one hundred sections, which permits the user to trace any specific drug by using the bulk drug number, appearing as the last portion of the section number, to reference each dosage form.

The following table shows the relationship of the CFR section numbers under the former Subchapter C to their redesignation reflected in the new Parts 430 through 460:

Old Section	*New Section*
141.1(a) and (b)	431.5
141.1(c) and (d)	436.1
141.1a	436.2
141.2	436.20
141.3	436.31
141.4	436.32
141.5	436.33
141.7	436.35
141.101	436.100
141.102	436.101
141.103	436.102
141.104	436.103
141.107	436.104
141.110	436.105
141.111	436.106
141.501	436.200
141.502	436.201
141.503	436.202
141.504	436.203
141.506	436.204
141.507	436.205
141.508	436.206
141.510	436.207
141.511	436.208
141.515	436.209
141.520	436.210
141.521	436.211
141.522	436.300
141.540	436.212
141.544	436.213
141.550	436.308
141.553	436.400
141.554	436.305
141.555	436.301
141.556	436.310
141.570	436.306
141.571	436.302
141.572	436.307
141.573	436.303
141.574	436.304
141.580	436.309
141a.1	440.80a(b), 440.280a
141a.2	440.80a(b), 440.280a
141a.3	440.80a(b), 440.280a
141a.4	440.80a(b), 440.280a
141a.5	440.80a(b), 440.280a
141a.6	440.80a(b), 440.280a
141a.7	436.500
141a.9	440.180a(b)
141a.10	440.73(b)
141a.16	440.153(b)
141a.19	440.81(b), 440.281
141a.21	440.180d(b)
141a.23	440.280d(b)
141a.24	440.53a(b)
141a.25	440.253(b)
141a.26	440.74a(b)
141a.27	440.274a(b)
141a.29	440.274b(b)
141a.30	440.63a(b)
141a.31	440.563(b)
141a.32	436.503
141a.33	440.180f(b)
141a.37	436.504
141a.42	440.280c(b)
141a.43	440.65a(b)
141a.44	440.265a(b)
141a.45	440.265b(b)
141a.47	440.55a(b)
141a.48	440.155c(b)
141a.49	436.505
141a.51	440.61a(b)
141a.52	440.261(b)
141a.54	440.255b(b)
141a.55	436.506
141a.56	440.59a(b)
141a.57	440.259(b)
141a.61	436.507
141a.62	440.255c(b)
141a.63	436.508
141a.64	440.180b(b)
141a.66	440.174(b)
141a.69	440.180e(b)
141a.70	440.155a(b)
141a.72	440.60a(b)
141a.73	440.160(b)
141a.75	440.66(b)
141a.76	440.166(b)
141a.77	440.180c(b)
141a.78	440.255a(b)
141a.81	440.71(b)
141a.82	440.171b(b)
141a.83	440.57a(b)
141a.84	440.155b(b)
141a.85	440.171a(b)
141a.86	436.509
141a.87	440.157(b)
141a.95	436.510
141a.98	436.511
141a.109	436.512
141a.130	440.173b(b)
141b.101	444.70a(b)(1), 444.270a
141b.102	444.70a(b)(2), 444.270a
141b.103	444.70a(b)(3), 444.270a
141b.104	444.70a(b)(4), 444.270a
141b.105	444.70a(b)(5), 444.270a
141b.106	444.70a(b)(6), 444.270a
141b.107	444.570a(b)
141b.108	444.10a(b)
141b.110	444.570b
141b.111 human provisions only	444.270b(b)
141b.112	444.170a(b)
141b.114	444.570c(b)
141b.115	444.470a(b)
141b.121	444.72a(b)
141b.139	444.270c(b)
141c.201	446.10a(b), 446.210
141c.202	446.510a(b), 446.310b
141c.203	436.513
141c.204	446.110b(b)
141c.205	446.510b(b)
141c.206	446.310a(b)
141c.207	446.110a(b)
141c.208	446.481(b)
141c.212	446.610(b), 446.710
141c.217	446.111(b)
141c.218	446.81a(b), 446.281a
141c.220	446.80(b)
141c.221	446.281b(b)
141c.222	446.181c(b)
141c.224	446.181b(b)
141c.225	446.181a(b)
141c.229	446.180a(b)
141c.230	436.514
141c.231	436.515
141c.232	446.82(b)
141c.236	446.180b(b)
141c.242	436.516
141c.244	446.581a(b)
141c.246	446.581b(b)
141c.247	446.910
141c.259	446.116d(b)
141c.263	446.115c(b)
141c.271	446.116b(b)
141d.301	455.10b(b)
141d.303	455.310c(b), 455.510a
141d.304	455.310b(b)
141d.308	455.410(b), 455.510b
141d.313	455.310d(b), 455.510c

Old Section	New Section
141e.401	448.10a(b), 448.210
141e.402	448.510a(b), 448.310c
141e.403 human provisions only	448.110a(b)
141e.407	448.510b(b)
141e.408	448.310a(b)
141e.409	448.510c(b)
141e.410	436.517
141e.411	448.510d(b)
141e.412	448.110b(b)
141e.418	448.13(b)
141e.422	448.510e(b)
141e.430	448.510f(b)
141e.432	448.410(b)
141e.433	448.310b(b)
141e.434	448.910(b)
141e.435	448.913(b)
144.1	433.1
144.2	433.2
144.3	433.12
144.4	433.14
144.5	433.15
144.6	433.16
144.7	433.13
144.8	433.17
144.9	433.24
144.10	433.22
144.12	433.23
144.14	433.21
144.15	433.3
144.16	433.25
144.17	433.20
144.23	433.30
145.1	430.3
145.2	430.4
145.3	430.5
145.4	430.6
145.31	430.10
145.32	430.11
146.1	430.30
146.2 except (b) (3) and (4)	431.1
146.2(b) (3) and (4)	431.16
146.3	431.10
146.4	431.11
146.5	431.61
146.6	431.51
146.7	431.62
146.8	431.53
146.9	431.20
146.10	431.17
146.11	432.20
146.12	431.12
146.13	431.50
146.14	431.60
146.15	431.52
146a.24 except (c) (2)	440.80a(a), 440.280a
146a.27 except (c) (2)	440.180a(a)
146a.28 except (c) (2)	440.180e(a)
146a.34 except (c) (2)	440.153(a)
146a.37	440.81(a), 440.281
146a.39 except (c) (2)	440.174(a)
146a.41 except (c) (2)	4440.280d(a)
146a.42	440.53a(a)
146a.43 except (c) (2)	440.253(a)
146a.44	440.74a(a)
146a.45 except (c) (2)	440.274a(a)
146a.47 except (c) (2)	440.274b(a)
146a.48	440.63a(a)
146a.49 except (c) (2)	440.563(a)
146a.51 except (c) (2)	440.180f(a)
146a.53	440.180d(a)
146a.61	440.73(a)
146a.63	440.280c(a)
146a.64	440.65a(a)
146a.65 except (c) (2)	440.265a(a)
146a.66 except (c) (2)	440.265b(a)
146a.68	440.55a(a)
146a.69 except (c) (2)	440.155c(a)
146a.74	440.61a(a)
146a.75 except (c) (2)	440.261(a)
146a.77 except (c) (2)	440.255b(a)
146a.79	440.59a(a)
146a.80 except (c) (2)	440.259(a)
146a.86	440.255c(a)
146a.88	440.180b(a)
146a.92	440.155a(a)
146a.94	440.60a(a)
146a.95 except (c) (2)	440.160(a)
146a.97	440.66(a)
146a.98 except (c) (2)	440.166(a)
146a.99	440.180c(a)
146a.100	440.255a(a)
146a.103	440.71(a)
146a.104 except (c) (2)	440.171b
146a.105	440.57a(a)
146a.106	440.155b(a)
146a.107	440.171a(a)
146a.109	440.157(a)
146a.126	440.173b(a)
146b.101 except (c) (2)	444.70a(a), 444.270(a)
146b.102 except (c) (2)	444.570a(a)
146b.103	444.10a(a)
146b.105 except (c) (2)	444.570b(a)
146b.106 human provisions only	444.270b(a)
146b.107 except (c) (2)	444.170a(a)
146b.109	444.570c(a)
146b.110 except (c) (2)	444.470a(a)
146b.116	444.72a(a)
146b.134	444.270c(a)
146c.201 except (c) (2)	446.10(a), 446.210
146c.202	446.510a(a), 446.310b
146c.204 except (c) (2)	446.110b(a)
146c.205 except (c) (2)	446.510b(a)
146c.206 except (c) (2)	446.310a(a)
146c.207	446.110a(a)
146c.208 except (c) (2)	446.481(a)
146c.212 except (c) (2)	446.610(a), 446.710
146c.217 except (c) (2)	446.111(a)
146c.218	446.81a(a), 446.281a
146c.220	446.80(a)
146c.221	446.281b(a)
146c.222 except (c) (2)	446.181c(a)
146c.224	446.181b(a)
146c.225	446.181a(a)
146c.229	446.180a(a)
146c.232	446.82(a)
146c.236	446.180b(a)
146c.244 except (c) (2)	446.581a(a)
146c.246	446.581b(a)
146c.247	446.910(a)
146c.259	446.116d(a)
146c.263	446.115c(a)
146c.271	446.116b(a)
146d.301	455.10b(a)
146d.303 except (c) (2)	455.310c(a), 455.510a
146d.304 except (c) (2)	455.310b(a)
146d.308 except (c) (2)	455.410(a), 455.510b
146d.313	455.310d(a), 455.510c
146e.401 except (c) (2)	448.10a(a), 448.210
146e.402 except (c) (2), (3), and (4)	448.510a(a), 448.310c
146e.403 human provisions only	448.110a(a)
146e.407	448.510b(a)
146e.408 except (c) (2)	448.310a(a)
146e.409	448.510c(a)
146e.411	448.510d(a)
146e.412	448.110b(a)
146e.418	448.13(a)
146e.422	448.510e(a)
146e.430 except (c) (2)	448.510f(a)
146e.432	448.410(a)
146e.433	448.310b(a)
146e.434	448.910(a)
146e.435	448.913(a)
147.1	460.6
147.2	460.1
147.5	460.38
147.6	460.15
147.7	460.47
147.8	460.25
147.9	460.42
147.10	460.66
147.11	460.79
147.12	460.89
147.13	460.93
147.14	460.70
147.15	460.75
147.16	460.58
147.17	460.33
147.18	460.28
147.19	460.55
147.20	460.64
147.21	460.86
148.2	432.1
148.3 except (d)	432.5
148.4	432.9
148a.1	455.3
148a.2	455.503a
148b.1	449.4
148b.1a	449.4a
148b.2	449.204
148b.3	449.504c
148b.4	449.504a
148b.5	449.504b
148b.6	449.104
148c.1	448.21
148c.2	448.421
148c.3	448.121
148c.4	448.20a, 448.220a
148c.5	448.220b
148c.6	448.321
148d.1	455.20
148d.2	455.120
148e.1	452.10
148e.2	452.20
148e.3	452.30a
148e.5	452.15
148e.6	452.35
148e.7	452.25
148e.7a	452.25a
148e.8	452.40
148e.10	452.125c
148e.11	452.120a
148e.12	452.115d
148e.13	452.125b
148e.14	452.510a
148e.15	452.510b
148e.17	452.115b
148e.18	452.225
148e.22	452.115e
148e.23	452.120b
148e.24	452.110b
148e.25	452.232
148e.26	452.110a
148e.27	452.135a
148e.29	452.125a
148e.30	452.115f
148e.31	452.310
148e.34	452.115c
148e.35	452.710
148e.36	452.115a
148e.37	452.135b
148f.1	448.25
148g.1	449.20
148g.2	449.120a
148g.3	449.120b
148g.4	449.120c
148h.1	444.30
148h.1a	444.30a
148h.2	444.230
148h.3	444.130
148i.1	444.42a
148i.2	444.44
148i.3	444.542a, 444.342e, 444.442d
148i.4	444.542b
148i.5	444.142a
148i.8	444.442a
148i.9	444.342a
148i.10	444.542c
148i.14	444.5421
148i.15	444.342d
148i.16	444.542e
148i.18	444.442c
148i.19	444.642a

Old Section	New Section
148i.20	444.342b
148i.22	444.342a
148i.23	444.342c
148i.24	444.442b
148i.25	444.542h
148i.26	444.542f, 444.342f
148i.27	444.542g
148i.30	444.542k
148i.30a	444.542l
148i.30b	444.542m
148i.31	444.42b
148i.32	444.40
148i.33	444.540a
148i.34	444.542j
148i.39	444.342g, 444.442e
148i.46	444.942b
148i.47	444.540b
148i.49	444.542d
148i.50	444.142b
148j.1	455.51
148j.1a	455.51a
148j.2	455.50
148j.3	455.151a
148j.5	455.150
148j.6	455.251
148j.7	455.251b
148k.1	449.50
148k.2	449.550a
148k.3	449.550c
148k.4	449.550f
148k.5	449.550g
148k.6	449.150c
148k.7	449.150a
148k.8	449.550d
148k.9	449.550e
148k.10	449.450
148k.11	449.650
148k.12	449.150b
148k.13	449.550b
148m.2	452.75
148m.4	452.175a
148m.7	452.175b
148m.8	452.175c
148m.9	452.175d
148n.1	446.65a
148n.2	446.67a
148n.3	446.66
148n.4	446.165b
148n.5	446.265
148n.6	446.165a
148n.8	446.165d
148n.9	446.165e
148n.10	446.165c
148n.11	446.267
148n.12	446.167
148n.17	446.367a
148n.18	446.367d
148n.19	446.567a
148n.20	446.567b
148n.21	446.367e, 446.467b
148n.22	446.367b
148n.23	446.367c, 446.467c
148n.24	446.166
148n.25	446.567d
148n.26	446.567e
148n.27	446.567c
148n.28	446.467a
148n.29	446.667
148o.1	444.50
148o.2	444.150a
148o.3	444.150b
148p.1	448.30a
148p.4	448.430
148p.8	448.930a
148p.9	448.930b
148q.1	444.20a
148q.1a	444.20
148q.2	444.520a
148q.3	444.520b
148q.4	444.220
148q.5	444.320a
148q.6	444.320b
148r.1	448.75
148s.1	455.85a, 455.285a
148s.2	455.85
148s.11	455.185
148t.1	448.80a, 448.280
148u.1	450.20a
148u.2	450.220
148v.1	449.10
148v.2	449.610b
148v.3	449.610a
148v.4	449.610c
148w.1	442.25a, 442.225
148w.2	442.23a, 442.223
148w.3	442.21
148w.4	442.121a
148w.5	442.121b
148w.6	442.27
148w.7	442.127c
148w.8	442.127b
148w.9	442.127a
148w.10	442.29a, 442.229
148w.11	442.11a, 442.211
148x.1	453.30
148x.1a	453.30a
148x.2	453.130a
148x.3	453.230
148x.5	453.130b
148y.1	446.50
148y.2	446.150a
148y.3	446.150b
148z.1	446.20
148z.1a	446.20a
148z.2	446.21
148z.3	446.120
148z.4	446.121
148z.5	446.220
149a.1	440.19
149a.2	440.19a, 440.219a
149a.11	440.119a
149a.12	440.119b
149a.13	440.219b
149b.1	440.7
149b.2	440.7a
149b.3	440.5
149b.4	440.9a, 440.209
149b.11	440.107b
149b.12	440.107a
149b.13	440.105a
149b.14	440.105d
149b.15	440.107c
149b.16	440.105c
149b.18	440.105b
149b.20	440.207
149b.25	440.107d
149c.1	440.25
149c.1a	440.29a, 440.229a
149c.1b	440.29
149c.2	440.229b
149c.3	440.129
149c.4	440.125a
149c.5	440.125b
149d.1	440.41
149d.2	440.41a
149d.11	440.141b
149d.12	440.241
149d.13	440.141c
149d.14	440.141a
149e.1	440.49
149e.2	440.49a
149e.11	440.149a
149e.12	440.149b
149e.13	440.249
149f.1	440.36a
149f.11	440.236
149g.11	440.280b
149h.3	440.69
149h.11	440.173a
149h.12	440.169b
149h.13	440.169a
149j.1	440.15
149j.11	440.115a
149j.12	440.115b
149k.1	440.82
149k.11	440.182a
149k.12	440.182b
149u.1	453.20
149u.2	453.120
149u.3	453.21
149u.4	453.22a
149u.12	453.121a
149u.13	453.222
149u.14	453.121b
149v.1	450.40
149v.2	450.240
149w.1	440.51
149w.2	440.151
149y.1	440.13a, 440.213
149y.2	440.11
149y.11	440.111
150b.1	446.16
150b.2	446.15
150b.11	446.116c
150b.12	446.116a
150b.13	446.115b
150b.14	446.115a
150d.1	446.75a
150d.2	446.76a
150d.11	446.275a
150d.12	446.275b
150d.13	446.276a
150d.14	446.276b
150g.1	446.60
150g.11	446.160b
150g.12	446.266
150g.13	446.160c
150g.14	446.160a
151a.1	455.70
151a.11	455.170
151b.3	448.13a
151b.22	448.313
151c.1	455.10a
151c.2	455.10
151c.3	455.11
151c.4	455.12a
151c.11	455.110
151c.13	455.210
151c.15	455.310a
151c.17	455.111
151g.1	448.15a, 448.215
151h.1	455.80a, 455.280a
151j.1	450.45
151j.11	450.245

The changes being made are nonsubstantive in nature and for this reason notice and public procedure are not prerequisites to this promulgation. For the convenience of the user, the entire text of Parts 430 through 460 of Subchapter D are set forth below.

Dated: May 24, 1974.

SAM D. FINE,
Associate Commissioner for Compliance.

Therefore 21 CFR is amended by redesignating the antibiotic drug provisions for human use under Parts 141 through 151j of Subchapter C—Drugs as Parts 430 through 460 of Subchapter D—Drugs for Human Use, and republished to read as follows:

Part
430 Antibiotic Drugs: General.
431 Certification of Antibiotic Drugs.
432 Packaging and Labeling of Antibiotic Drugs.
433 Exemptions from Antibiotic Certification and Labeling Requirements.
436 Test and Methods of Assay of Antibiotic and Antibiotic-Containing Drugs.
440 Penicillin Antibiotic Drugs.
442 Cepha Antibiotic Drugs.
444 Oligosaccharide Antibiotic Drugs.
446 Tetracycline Antibiotic Drugs.
448 Peptide Antibiotic Drugs.
449 Antifungal Antibiotic Drugs.
450 Antitumor Antibiotic Drugs.
452 Marcolide Antibiotic Drugs.
453 Lincomycin Antibiotic Drugs.
455 Certain Other Antibiotic Drugs.
460 Antibiotic Drugs Intended for Use in Laboratory Diagnosis of Disease.

PART 430—ANTIBIOTIC DRUGS: GENERAL

Subpart A—General Provisions

Sec.
430.3 Definitions applicable to all certifiable antibiotic drugs.
430.4 Definitions of antibiotic substances.
430.5 Definitions of master and working standards.
430.6 Definitions of the terms "unit" and "microgram" as applied to antibiotic substances.

Subpart B—Procedures for Antibiotic Drugs Affected by Drug Amendments of 1962

430.10 Antibiotic drugs not subject to certification prior to May 1, 1963.
430.11 Certification, release, or exemption of antibiotic-containing drugs.

Subpart C—Administrative Procedures

430.20 Procedure for the issuance, amendment, or repeal of regulations.

AUTHORITY: Sec. 507, 701(a), 59 Stat. 463, as amended, 52 Stat. 1055 (21 U.S.C. 357, 371 (a)).

Subpart A—General Provisions

§ 430.3 Definitions applicable to all certifiable antibiotic drugs.

(a) The definitions and interpretations contained in section 201 of the Federal Food, Drug, and Cosmetic Act shall be applicable to such terms when used in the regulations in this chapter covering the certification of antibiotic and antibiotic-containing drugs.

(b) The term "Commissioner" means the Commissioner of Food and Drugs and any other officer of the Food and Drug Administration whom he may designate to act in his behalf for the purpose of the regulations for the certification of antibiotic and antibiotic-containing drugs.

(c) The term "act" means the Federal Food, Drug, and Cosmetic Act and amendments thereto. (52 Stat. 1040 et seq.; 21 U.S.C. 301–392).

(d) The term "U.S.P." means the official Pharmacopeia of the United States, including supplements thereto. The term "N.F." means the official National Formulary, including supplements thereto.

(e) The term "batch" means a specific homogeneous quantity of a drug.

(f) The term "batch mark" means an identifying mark or other identifying device assigned to a batch by the manufacturer or packer thereof.

(g) The term "manufacture" does not include the use of a drug as an ingredient in compounding any prescription issued by a practitioner licensed by law to administer such drug.

§ 430.4 Definitions of antibiotic substances.

(a) The following are definitions of antibiotic substances:

(1) *Penicillin.* Each of the several antibiotic substances (e.g., penicillin F, penicillin G, penicillin X) produced by the growth of *Penicillium notatum* or *Penicillium chrysogenum*, and each of the same substances produced by any other means, is a kind of penicillin.

(2) *Streptomycin.* Each of the several antibiotic substances produced by the growth of *Streptomyces griseus*, and each of the same substances produced by any other means, is a kind of streptomycin.

(3) *Dihydrostreptomycin.* Each of the antibiotic substances produced by hydrogenation of streptomycin, and each of the same substances produced by any other means, is a kind of dihydrostreptomycin.

(4) *Chlortetracycline.* Each of the several antibiotic substances produced by the growth of *Streptomyces aureofaciens*, and each of the same substances produced by any other means is a kind of chlortetracycline.

(5) *Tetracycline.* Each of the several antibiotic substances produced by the hydrogenation of chlortetracycline, and each of the same substances produced by any other means, is a kind of tetracycline.

(6) *Chloramphenicol.* Each of the several antibiotic substances produced by the growth of *Streptomyces venezuelae*, and each of the same substances produced by any other means, is a kind of chloramphenicol.

(7) *Bacitracin.* Each of the several antibiotic substances produced by the growth of *Bacillus subtilis* var. Tracy, and each of the same substances produced by any other means, is a kind of bacitracin.

(8) *Amphomycin.* Each of the antibiotic substances produced by the growth of *Streptomyces canus*, and each of the same substances produced by any other means, is a kind of amphomycin.

(9) *Amphotericin.* Each of the antibiotic substances produced by the growth of *Streptomyces nodosus*, and each of the same substances produced by any other means, is a kind of amphortericin.

(10) *Colistin.* Each of the antibiotic substances produced by the growth of *Bacillus polymyxa* var. *colistinus*, and each of the same substances produced by any other means, is a kind of colistin.

(11) *Cycloserine.* Each of the antibiotic substances produced by the growth of *Streptomyces orchidaceus*, and each of the same substances produced by any other means, is a kind of cycloserine.

(12) *Erythromycin.* Each of the antibiotic substances produced by the growth of *Streptomyces erythreus*, and each of the same substances produced by any other means, is a kind of erythromycin.

(13) *Gramicidin.* Each of the antibiotic substances produced by the growth of *Bacillus brevis*, and each of the same substances produced by any other means, is a kind of gramicidin.

(14) *Griseofulvin.* Each of the antibiotic substances produced by the growth of *Penicilliun patulum* or *Penicillium griseofulvum*, and each of the same substances produced by any other means, is a kind of griseofulvin.

(15) *Kanamycin.* Each of the antibiotic substances produced by the growth of *Streptomyces kanamyceticus*, and each of the same substances produced by any other means, is a kind of kanamycin.

(16) *Neomycin.* Each of the antibiotic substances produced by the growth of *Streptomyces fradiae*, and each of the same substances produced by any other means, is a kind of neomycin.

(17) *Novobiocin.* Each of the antibiotic substances produced by the growth of *Streptomyces niveus* (known also as *Streptomyces spheroides*), and each of the same substances produced by any other means, is a kind of novobiocin.

(18) *Nystatin.* Each of the antibiotic substances produced by the growth of *Streptomyces noursei*, and each of the same substances produced by any other means, is a kind of nystatin.

(19) *Oleandomycin.* Each of the antibiotic substances produced by the growth of *Streptomyces antibioticus*, and each of the same substances produced by any other means, is a kind of oleandomycin.

(20) *Troleandomycin.* Each of the antibiotic substances produced by the triacetylation of oleandomycin, and each of the same substances produced by any other means, is a kind of troleandomycin.

(21) *Oxytetracycline.* Each of the antibiotic substances produced by the growth of *Streptomyces rimosus*, and each of the same substances produced by any other means, is a kind of oxytetracycline.

(22) *Paromomycin.* Each of the antibiotic substances produced by the growth of *Streptomyces rimosus* var. *paromomycinus*, and each of the same substances produced by any other means, is a kind of paromomycin.

(23) *Polymyxin.* Each of the antibiotic substances produced by the growth of *Bachillus polymyxa*, and each of the same substances produced by any other means, is a kind of polymyxin.

(24) *Mithramycin.* Each of the antibiotic substances produced by the growth of a variant of *Streptomyces plicatus*, and each of the same substances produced by any other means, is a kind of mithramycin.

(25) *Tyrothricin.* Each of the mixtures of antibiotic substances produced by the growth of *Bacillus brevis*, and each of the same mixtures of substances produced by any other means, is a kind of tyrothricin.

(26) *Vancomycin.* Each of the antibiotic substances produced by the growth of *Streptomyces orientalis,* and each of the same substances produced by any other means, is a kind of vancomycin.

(27) *Viomycin.* Each of the antibiotic substances produced by the growth of *Streptomyces puniceus* or *Streptomyces floridae* or *Actinomyces vinaceus,* and each of the same substances produced by any other means, is a kind of viomycin.

(28) *Gentamicin.* Each of the antibiotic substances produced by the growth of *Micromonospora purpurea,* and each of the same substances produced by any other means, is a kind of gentamicin.

(29) *Dactinomycin.* Dactinomycin is a specific kind of actinomycin produced by the growth of *Streptomyces parvullus* or the same antibiotic produced by any other means.

(30) *Candicidin.* Each of the heptaene antibiotic substances produced by the growth of *Streptomyces griseus* and each of the same substances produced by any other means is a kind of candicidin.

(31) *Cephalosporin.* Each of the antibiotic substances produced by the growth of *Cephalosporium acremonium,* and each of the same substances produced by any other means, is a kind of cephalosporin.

(32) *Lincomycin.* Each of the antibiotic substances produced by the growth of *Streptomyces lincolnensis* var. *lincolnensis,* and each of the same substances produced by any other means, is a kind of lincomycin.

(33) *Demeclocycline.* Each of the antibiotic substances produced by removal of the 6-methyl group from chlortetracycline, and each of the same substances produced by any other means, is a kind of demeclocycline.

(34) *Clindamycin.* Each of the antibiotic substances produced by the 7-chloro- substitution of the 7(R) hydroxyl group of lincomycin, and each of the same substances produced by any other means, is a kind of clindamycin.

(35) *Penicillamine.* Penicillamine is a hydrolysis product of penicillin.

(36) *Capreomycin.* Each of the antibiotic substances produced by the growth of *Streptomyces capreolus,* and each of the same substances produced by any other means, is a kind of capreomycin.

(37) *Rifamycin.* Each of the several antibiotic substances (e.g., rifamycin A, rifamycin B, rifamycin SV) produced by the growth of *Streptomyces mediterranei,* and each of the same substances produced by any other means, is a kind of rifamycin.

(38) *Spectinomycin.* Each of the antibiotic substances produced by the growth of *Streptomyces spectabilis,* and each of the same substances produced by any other means, is a kind of spectinomycin.

(39) *Mitomycin.* Mitomycin is the antibiotic substance produced by the growth of *Streptomyces caespitosus,* and each of the same substances produced by any other means is a kind of mitomycin.

§ 430.5 Defintions of master and working standards.

(a) *Master standards*—(1) *Penicillin and salts of penicillin*—(i) *Penicillin G.* The term "penicillin G master standard" means a specific lot of crystalline penicillin G that is designated by the Commissioner as the standard of comparison in determining the potency of the penicillin G working standard.

(ii) *Penicillin O.* The term "penicillin O master standard" means a specific lot of crystalline potassium penicillin O that is designated by the Commissioner as the standard of comparison in determining the potency of the penicillin O working standard.

(iii) *Phenoxymethyl penicillin.* The term "phenoxymethyl penicillin master standard" means a specific lot of crystalline phenoxymethyl penicillin that is designated by the Commissioner as the standard of comparison in determining the potency of the phenoxymethyl penicillin working standard.

(iv) *L-Phenethicillin.* The term "L-phenethicillin master standard" means a specific lot of crystalline L-phenethicillin that is designated by the Commissioner as the standard of comparison in determining the potency of the L-phenethicillin working standard.

(v) *D-Phenethicillin.* The term "D-phenethicillin master standard" means a specific lot of crystalline D-phenethicillin that is designated by the Commissioner as the standard of comparison in determining the potency of the D-phenethicillin working standard.

(vi) *Methicillin.* The term "methicillin master standard" means a specific lot of crystalline methicillin that is designated by the Commissioner as the standard of comparison in determining the potency of the methicillin working standard.

(vii) *Oxacillin.* The term "oxacillin master standard" means a specific lot of crystalline oxacillin that is designated by the Commissioner as the standard of comparison in determining the potency of the oxacillin working standard.

(viii) *Ampicillin.* The term "ampicillin master standard" means a specific lot of crystalline ampicillin that is designated by the Commissioner as the standard of comparison in determining the potency of the ampicillin working standard.

(ix) *Nafcillin.* The term "nafcillin master standard" means a specific lot of crystalline nafcillin that is designated by the Commissioner as the standard of comparison in determining the potency of the nafcillin working standard.

(x) *Cloxacillin.* The term "cloxacillin master standard" means a specific lot of crystalline cloxacillin that is designated as the standard of comparison in determining the potency of the cloxacillin working standard.

(xi) *Dicloxacillin.* The term "dicloxacillin master standard" means a specific lot of dicloxacillin that is designated by the Commissioner as the standard of comparison in determining the potency of the dicloxacillin working standard.

(xii) The term "hetacillin working standard" means a specific lot of homogenous preparation of hetacillin.

(2) *Streptomycin.* The term "streptomycin master standard" means a specific lot of streptomycin that is designated by the Commissioner as the standard of comparison in determining the potency of the streptomycin working standard.

(3) *Dihydrostreptomycin.* The term "dihydrostreptomycin master standard" means a specific lot of crystalline dihydrostreptomycin that is designated by the Commissioner as the standard of comparison in determining the potency of the dihydrostreptomycin working standard.

(4) *Chlortetracycline.* The term "chlortetracyclin master standard" means a specific lot of crystalline chlortetracycline hydrochloride that is designated by the Commissioner as the standard of comparison in determining the potency of the chlortetracycline working standard.

(5) *Demeclocycline.* The term "demeclocycline master standard" means a specific lot of crystalline demeclocycline hydrochloride that is designated by the Commissioner as the standard of comparison in determining the potency of the demeclocycline working standard.

(6) *Tetracycline.* The term "tetracycline master standard" means a specific lot of crystalline tetracycline hydrochloride that is designated by the Commissioner as the standard of comparison in determining the potency of the tetracycline working standard.

(7) *Rolitetracycline.* The term "rolitetracycline master standard" means a specific lot of crystalline rolitetracycline that is designated by the Commissioner as the standard of comparison in determining the potency of the rolitetracycline working standard.

(8) *Chloramphenicol.* The term "chloramphenicol master standard" means a specific lot of crystalline chloramphenicol that is designated by the Commissioner as the standard of comparison in determining the potency of the chloramphenicol working standard.

(9) *Bacitracin.* The term "bacitracin master standard" means a specific lot of bacitracin that is designated by the Commissioner as the standard of comparison in determining the potency of the bacitracin working standard.

(10) *Amphomycin.* The term "amphomycin master standard" means a specific lot of amphomycin designated by the Commissioner as the standard of comparison in determining the potency of the amphomycin working standard.

(11) *Amphotericin.* The term "amphotericin A master standard" means a specific lot of amphotericin A designated by the Commissioner as the standard of comparison in determining the potency of the amphotericin A working standard. The term "amphotericin B master standard" means a specific lot of amphotericin B designated by the Commis-

sioner as the standard of comparison in determining the potency of the amphotericin B working standard.

(12) *Colistin.* The term "colistin master standard" means a specific lot of colistin designated by the Commissioner as the standard of comparison in determining the potency of the colistin working standard.

(13) *Colistimethate.* The term "colistimethate master standard" means a specific lot of colistimethate designated by the Commissioner as the standard of comparison in determining the potency of the colistimethate working standard.

(14) *Cycloserine.* The term "cycloserine master standard" means a specific lot of cycloserine designated by the Commissioner as the standard of comparison in determining the potency of the cycloserine working standard.

(15) *Erythromycin.* The term "erythromycin master standard" means a specific lot of erythromycin designated by the Commissioner as the standard of comparison in determining the potency of the erythromycin working standard.

(16) *Gramicidin.* The term "gramicidin master standard" means a specific lot of gramicidin designated by the Commissioner as the standard of comparison in determining the potency of the gramicidin working standard.

(17) *Griseofulvin.* The term "griseofulvin master standard" means a specific lot of griseofulvin designated by the Commissioner as the standard of comparison in determining the potency of the griseofulvin working standard.

(18) *Kanamycin.* The term "kanamycin master standard" means a specific lot of kanamycin designated by the Commissioner as the standard of comparison in determining the potency of the kanamycin working standard.

(19) *Neomycin.* The term "neomycin master standard" means a specific lot of neomycin designated by the Commissioner as the standard of comparison in determining the potency of the neomycin working standard.

(20) *Novobiocin.* The term "novobiocin master standard" means a specific lot of novobiocin designated by the Commissioner as the standard of comparison in determining the potency of the novobiocin working standard.

(21) *Nystatin.* The term "nystatin master standard" means a specific lot of nystatin designated by the Commissioner as the standard of comparison in determining the potency of the nystatin working standard.

(22) *Oleandomycin.* The term "oleandomycin master standard" means a specific lot of oleandomycin designated by the Commissioner as the standard of comparison in determining the potency of the oleandomycin working standard.

(23) *Oxytetracycline.* The term "oxytetracycline master standard" means a specific lot of oxytetracycline designated by the Commissioner as the standard of comparison in determining the potency of the oxytetracycline working standard.

(24) *Paromomycin.* The term "paromomycin master standard" means a specific lot of paromomycin designated by the Commissioner as the standard of comparison in determining the potency of the paromomycin working standard.

(25) *Polymyxin B.* The term "polymyxin B master standard" means a specific lot of polymyxin B designated by the Commissioner as the standard of comparison in determining the potency of the polymyxin B working standard.

(26) [Reserved]

(27) *Vancomycin.* The term "vancomycin master standard" means a specific lot of vancomycin designated by the Commissioner as the standard of comparison in determining the potency of the vancomycin working standard.

(28) *Viomycin.* The term "viomycin master standard" means a specific lot of viomycin designated by the Commissioner as the standard of comparison in determining the potency of the viomycin working standard.

(29) *Troleandomycin.* The term "troleandomycin master standard" means a specific lot of troleandomycin designated by the Commissioner as the standard of comparison in determining the potency of the troleandomycin working standard.

(30) *Gentamicin.* The term "gentamicin master standard" means a specific lot of gentamicin designated by the Commissioner as the standard of comparison in determining the potency of the gentamicin working standard.

(31) *Dactinomycin.* The term "dactinomycin master standard" means a specific lot of dactinomycin designated by the Commissioner as the standard of comparison in determining the potency of the dactinomycin working standard.

(32) *Candicidin.* The term "candicidin master standard" means a specific lot of candicidin that is designated by the Commissioner as the standard of comparison in determining the potency of the candicidin working standard.

(33) *Cephalothin.* The term "cephalothin master standard" means a specific lot of cephalothin designated by the Commissioner as the standard of comparison in determining the potency of the cephalothin working standard.

(34) *Lincomycin.* The term "lincomycin master standard" means a specific lot of lincomycin designated by the Commissioner as the standard of comparison in determining the potency of the lincomycin working standard.

(35) *Methacycline.* The term "methacycline master standard" means a specific lot of methacycline designated by the Commissioner as the standard of comparison in determining the potency of the methacycline working standard.

(36) *Doxycycline.* The term "doxycycline master standard" means a specific lot of α-6-deoxyoxytetracycline designated by the Commissioner as the standard of comparison in determining the potency of the doxycycline working standard.

(37) *Cephaloridine.* The term "cephaloridine master standard" means a specific lot of cephaloridine that is designated by the Commissioner as the standard of comparison in determining the potency of the cephaloridine working standard.

(38) *Mithramycin.* The term "mithramycin master standard" means a specific lot of mithramycin designated by the Commissioner as the standard of comparison in determining the potency of the mithramycin working standard.

(39) *Clindamycin.* The term "clindamycin master standard" means a specific lot of clindamycin designated by the Commissioner as the standard of comparison in determining the potency of the clindamycin working standard.

(40) *Cephaloglycin.* The term "cephaloglycin master standard" means a specific lot of cephaloglycin designated by the Commissioner as the standard of comparison in determining the potency of the cephaloglycin working standard.

(41) *Carbenicillin.* The term "carbenicillin master standard" means a specific lot of carbenicillin designated by the Commissioner as the standard of comparison in determining the potency of the carbenicillin working standard.

(42) *Cephalexin.* The term "cephalexin master standard" means a specific lot of cephalexin that is designated by the Commissioner as the standard of comparison in determining the potency of the cephalexin working standard.

(43) *Penicillamine.* The term "penicillamine master standard" means a specific lot of penicillamine that is designated by the Commissioner as the standard of comparison in determining the penicillamine content of the penicillamine working standard.

(44) *Capreomycin.* The term "capreomycin master standard" means a specific lot of capreomycin designated by the Commissioner as the standard of comparison in determining the potency of the capreomycin working standard.

(45) *Rifampin.* The term "rifampin master standard" means a specific lot of rifampin designated by the Commissioner as the standard of comparison in determining the potency of the rifampin working standard.

(46) *Minocycline.* The term "minocycline master standard" means a specific lot of minocycline designated by the Commissioner as the standard of comparison in determining the potency of the minocycline working standard.

(47) *Spectinomycin.* The term "spectinomycin master standard" means a specific lot of spectinomycin designated by the Commissioner as the standard of comparison in determining the potency of the spectinomycin working standard.

(48) *Clindamycin palmitate hydrochloride.* The term "clindamycin palmitate hydrochloride master standard" means a specific lot of clindamycin palmitate hydrochloride designated by the Commissioner as the standard of comparison in determining the potency of the clindamycin palmitate hydrochloride working standard.

(49) *Carbenicillin indanyl.* The term "carbenicillin indanyl master standard" means a specific lot of carbenicillin indanyl designated by the Commissioner as the standard of comparison in determining the potency of the carbenicillin indanyl working standard.

(50) *Cephapirin.* The term "cephapirin master standard" means a specific lot of cephapirin that is designated by the Commissioner as the standard of com-

parison in determining the potency of the cephapirin working standard.

(51) *Cefazolin.* The term "cefazolin master standard" means a specific lot of cefazolin that is designated by the Commissioner as the standard of comparison in determining the potency of the cefazolin working standard.

(52) *Mitomycin.* The term "mitomycin master standard" means a specific lot of crystalline mitomycin that is designated by the Commissioner as the standard of comparison in determining the potency of the mitomycin working standard.

(b) *Working standards.* The potency or purity of each preparation has been determined by comparison with its master standard, and each has been designated by the Commissioner as working standards for use in determining the potency or purity of antibiotic substances subject to the regulations in this chapter.

(1) *Penicillin.* (i) The term "penicillin G working standard" means a specific lot of a homogeneous preparation of penicillin G.

(ii) The term "penicillin O working standard" means a specific lot of a homogeneous preparation of penicillin O.

(iii) The term "phenoxymethyl penicillin working standard" means a specific lot of a homogeneous preparation of penicillin.

(iv) The term "L-phenethicillin working standard" means a specific lot of a homogeneous preparation of L-phenethicillin. The term "D-phenethicillin working standard" means a specific lot of a homogeneous preparation of D-phenethicillin.

(v) The term "methicillin working standard" means a specific lot of a homogeneous preparation of methicillin.

(vi) The term "oxacillin working standard" means a specific lot of a homogeneous preparation of oxacillin.

(vii) The term "ampicillin working standard" means a specific lot of a homogeneous preparation of ampicillin.

(viii) The term "nafcillin working standard" means a specific lot of a homogeneous preparation of nafcillin.

(ix) The term "cloxacillin working standard" means a specific lot of a homogeneous preparation of cloxacillin.

(x) The term "procaine penicillin G working standard" means a specific lot of a homogeneous preparation of procaine penicillin G.

(xi) The term "dicloxacillin working standard" means a specific lot of a homogeneous preparation of dicloxacillin.

(2) *Amphotericin A.* The term "amphotericin A working standard" means a specific lot of a homogeneous preparation of amphotericin A.

(3) *Amphotericin B.* The term "amphotericin B working standard" means a specific lot of a homogeneous preparation of amphotericin B.

(4) *Streptomycin.* The term "streptomycin working standard" means a specific lot of a homogeneous preparation of streptomycin.

(5) *Dihydrostreptomycin.* The term "dihydrostreptomycin working standard" means a specific lot of a homogeneous preparation of dihydrostreptomycin.

(6) *Chlortetracycline.* The term "chlortetracycline working standard" means a specific lot of a homogeneous preparation of chlortetracycline.

(7) *Demeclocycline.* The term "demeclocycline working standard" means a specific lot of a homogeneous preparation of demeclocycline.

(8) *Tetracycline.* The term "tetracycline working standard" means a specific lot of a homogeneous preparation of tetracycline.

(9) *Rolitetracycline.* The term "rolitetracyline working standard" means a specific lot of a homogeneous preparation of rolitetracycline.

(10) *Chloramphenicol.* The term "chloramphenicol working standard" means a specific lot of a homogeneous preparation of chloramphenicol.

(11) *Bacitracin.* The term "bacitracin working standard" means a specific lot of a homogeneous preparation of bacitracin.

(12) *Amphomycin.* The term "amphomycin working standard" means a specific lot of a homogeneous preparation of amphomycin.

(13) *Colistin.* The term "colistin working standard" means a specific lot of a homogeneous preparation of colistin.

(14) *Colistimethate.* The term "colistimethate working standard" means a specific lot of a homogeneous preparation of colistimethate.

(15) *Cycloserine.* The term "cycloserine working standard" means a specific lot of a homogeneous preparation of cycloserine.

(16) *Erythromycin.* The term "erythromycin working standard" means a specific lot of a homogeneous preparation of erythromycin.

(17) *Gramicidin.* The term "gramicidin working standard" means a specific lot of a homogeneous preparation of gramicidin.

(18) *Griseofulvin.* The term "griseofulvin working standard" means a specific lot of a homogeneous preparation of griseofulvin.

(19) *Kanamycin.* The term "kanamycin working standard" means a specific lot of a homogeneous preparation of kanamycin.

(20) *Neomycin.* The term "neomycin working standard" means a specific lot of a homogeneous preparation of neomycin.

(21) *Novobiocin.* The term "novobiocin working standard" means a specific lot of a homogeneous preparation of novobiocin.

(22) *Nystatin.* The term "nystatin working standard" means a specific lot of a homogeneous preparation of nystatin.

(23) *Oleandomycin.* The term "oleandomycin working standard" means a specific lot of a homogeneous preparation of oleandomycin.

(24) *Troleandomycin.* The term "troleandomycin working standard" means a specific lot of a homogeneous preparation of troleandomycin.

(25) *Oxytetracycline.* The term "oxytetracycline working standard" means a specific lot of a homogeneous preparation of oxytetracycline.

(26) *Paromomycin.* The term "paromomycin working standard" means a specific lot of a homogeneous preparation of paromomycin.

(27) *Polymyxin B.* The term "polymyxin B working standard" means a specific lot of a homogeneous preparation of polymyxin B.

(28) *Vancomycin.* The term "vancomycin working standard" means a specific lot of a homogeneous preparation of vancomycin.

(29) *Viomycin.* The term "viomycin working standard" means a specific lot of a homogeneous preparation of viomycin.

(30) *Gentamicin.* The term "gentamicin working standard" means a specific lot of a homogeneous preparation of gentamicin.

(31) *Dactinomycin.* The term "dactinomycin working standard" means a specific lot of a homogeneous preparation of dactinomycin.

(32) *Candicidin.* The term "candicidin working standard" means a specific lot of a homogeneous preparation of candicidin.

(33) *Cephalothin.* The term "cephalothin working standard" means a specific lot of a homogeneous preparation of cephalothin.

(34) *Lincomycin.* The term "lincomycin working standard" means a specific lot of a homogeneous preparation of lincomycin.

(35) *Methacycline.* The term "methacycline working standard" means a specific lot of homogeneous preparation of methacycline.

(36) *Doxycycline.* The term "doxycycline working standard" means a specific lot of homogeneous preparation of α-6-deoxyoxytetracycline.

(37) *Cephaloridine.* The term "cephaloridine working standard" means a specific lot of homogeneous preparation of cephaloridine.

(38) *Mithramycin.* The term "mithramycin working standard" means a specific lot of a homogeneous preparation of mithramycin.

(39) *Clindamycin.* The term "clindamycin working standard" means a specific lot of a homogeneous preparation of clindamycin.

(40) *Cephaloglycin.* The term "cephaloglycin working standard" means a specific lot of homogeneous preparation of cephaloglycin.

(41) *Carbenicillin.* The term "carbenicillin working standard" means a specific lot of homogeneous preparation of carbenicillin.

(42) *Cephalexin.* The term "cephalexin working standard" means a specific lot of a homogeneous preparation of cephalexin.

(43) *Penicillamine.* The term "penicillamine working standard" means a specific lot of a homogeneous preparation of penicillamine.

(44) *Capreomycin.* The term "capreomycin working standard" means a specific lot of a homogeneous preparation of capreomycin.

(45) *Rifampin.* The term "rifampin working standard" means a specific lot of a homogeneous preparation of rifampin.

(46) *Minocycline.* The term "minocycline working standard" means a specific lot of a homogeneous preparation of minocycline.

(47) *Spectinomycin.* The term "spectinomycin working standard" means a specific lot of a homogeneous preparation of spectinomycin.

(48) *Clindamycin palmitate hydrochloride.* The term "clindamycin palmitate hydrochloride working standard" means a specific lot of a homogeneous preparation of clindamycin palmitate hydrochloride.

(49) *Carbenicillin indanyl.* The term "carbenicillin indanyl working standard" means a specific lot of a homogeneous preparation of carbenicillin indanyl.

(50) *Cephapirin.* The term "cephapirin working standard" means a specific lot of a homogeneous preparation of cephapirin.

(51) *Cefazolin.* The term "cefazolin working standard" means a specific lot of a homogeneous preparation of cefazolin.

(52) *Mitomycin.* The term "mitomycin working standard" means a specific lot of a homogeneous preparation of mitomycin.

§ 430.6 Definitions of the terms "unit" and "microgram" as applied to antibiotic substances.

Unless it has been otherwise specified in the individual definitions in this section, the activity assigned to each "unit" or "microgram" is equivalent to an International Unit, if such has been defined by the World Health Organization.

(a) *"Unit"*—(1) *Penicillin*—(i) *Penicillin G.* The term "unit" applied to penicillin G means the penicillin activity (potency) contained in 0.600 microgram of the penicillin G master standard.

(ii) *Penicillin O.* The term "unit" applied to penicillin O means the penicillin activity (potency) contained in 0.6203 microgram of the penicillin O master standard after it is dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(iii) *Phenoxymethyl penicillin.* The term "unit" applied to phenoxymethyl penicillin means the penicillin activity (potency) contained in 0.590 microgram of the phenoxymethyl penicillin master standard.

(iv) *Phenethicillin.* The term "unit" applied to phenethicillin means the penicillin activity (potency) contained in 0.6775 microgram of the D-phenethicillin master standard or in 0.6803 microgram of the L-phenethicillin master standard.

(2) *Bacitracin.* The term "unit" applied to bacitracin means a bacitracin activity (potency) contained in 13.51 micrograms of the bacitracin master standard, except that when the activity (potency) of bacitracin is expressed in terms of its weight, as in the feed and drinking water of animals, 1 gram of activity is equivalent to 42,000 units.

(3) *Nystatin* The term "unit" applied to nystatin means the nystatin activity (potency) contained in 0.2817 microgram of the nystatin master standard when dried for 2 hours at 40° C. and a pressure of 5 millimeters or less.

(4) *Polymyxin B.* The term "unit" applied to polymyxin B means the polymyxin activity (potency) contained in 0.1274 microgram of the polymyxin B master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(b) *"Microgram"*—(1) *Streptomycin.* The term "microgram" applied to streptomycin means the streptomycin activity (potency) contained in 1.250 micrograms of the streptomycin master standard after it is dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(2) *Dihydrostreptomycin.* The term "microgram" applied to dihydrostreptomycin means the dihydrostreptomycin activity (potency) contained in 1.25 micrograms of the dihydrostreptomycin master standard after it is dried for 4 hours at 100° C. and a pressure of 50 microns or less.

(3) *Chlortetracycline.* The term "microgram" applied to chlortetracycline means the chlortetracycline activity (potency) contained in 1.0 microgram of the chlortetracycline master standard.

(4) *Demeclocycline.* The term "microgram" applied to demeclocycline means the demeclocycline activity (potency) contained in 1.0 microgram of the demeclocycline master standard after it is dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(5) *Tetracycline.* The term "microgram" applied to tetracycline means the tetracycline activity (potency) contained in 1.0 microgram of tetracycline master standard.

(6) *Rolitetracycline.* The term "microgram" applied to rolitetracycline means the rolitetracycline activity (potency) contained in 1.0 microgram of the rolitetracycline master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(7) *Chloramphenicol.* The term "microgram" applied to chloramphenicol means the chloramphenicol activity (potency) contained in 1.0 microgram of the chloramphenicol master standard.

(8) *Methicillin.* The term "microgram" applied to methicillin means the methicillin activity (potency) contained in 1.105 micrograms of the methicillin master standard.

(9) *Oxacillin.* The term "microgram" applied to oxacillin means the oxacillin activity (potency) contained in 1.111 micrograms of the oxacillin master standard.

(10) *Amphomycin.* The term "microgram" applied to amphomycin means the amphomycin activity (potency) contained in 0.9355 microgram of the amphomycin master standard when dried for 4 hours at 60° C. and a pressure of 5 millimeters or less.

(11) *Amphotericin A.* The term "microgram" applied to amphotericin A means the amphotericin A activity (potency) contained in 1.0 microgram of the amphotericin A master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(12) *Amphotericin B.* The term "microgram" applied to amphotericin B means the amphotericin B activity (potency) contained in 1.124 micrograms of the amphotericin B master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(13) *Colistin.* The term "microgram" applied to colistin means the colistin base activity (potency) contained in 1.495 micrograms of the colistin master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less. The numerical value of a microgram of colistin is not equivalent to the International Unit.

(14) *Colistimethate.* The term "microgram" applied to colistimethate means the activity (potency) calculated as colistin base that is contained in 1.938 micrograms of the colistimethate master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less. The numerical value of a microgram of colistimethate is not equivalent to the International Unit.

(15) *Cycloserine.* The term "microgram" applied to cycloserine means the cycloserine activity (potency) contained in 1.0 microgram of the cycloserine master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(16) *Erythromycin.* The term "micrograms" applied to erythromycin means the erythromycin base activity (potency) contained in 1.02 micrograms of the erythromycin master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(17) *Gramicidin.* The term "microgram" applied to gramicidin means the gramicidin activity (potency) contained in 1.0 microgram of the gramicidin master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(18) *Griseofulvin.* The term "microgram" applied to griseofulvin means the griseofulvin activity (potency) contained in 1.0 microgram of the griseofulvin master standard.

(19) *Kanamycin.* The term "microgram" applied to kanamycin means the kanamycin base activity (potency) contained in 1.299 micrograms of the kanamycin master standard.

(20) *Neomycin.* The term "microgram" applied to neomycin means the neomycin base activity (potency) contained in 1.429 micrograms of the neomycin master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(21) *Novobiocin.* The term "microgram" applied to novobiocin means the novobiocin acid activity (potency) contained in 1.033 micrograms of the novobiocin master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(22) *Oleandomycin.* The term "microgram" applied to oleandomycin means the oleandomycin base activity (potency) contained in 1.176 micrograms of the oleandomycin master standard.

(23) *Troleandomycin.* The term "microgram" applied to troleandomycin means the activity (potency), calculated as the molecular equivalent of the oleandomycin base, contained in 1.2315 micrograms of the troleandomycin master standard.

(24) *Oxytetracycline.* The "microgram" applied to oxytetracycline means the oxytetracycline base activity (potency) contained in 1.13 micrograms of the oxytetracycline master standard.

(25) *Paromomycin.* The term "microgram" applied to paromomycin means the paromomycin activity (potency) contained in 1.333 micrograms of the paromomycin master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(26) *Tyrothricin.* The term "microgram" applied to tyrothricin means the activity (potency) contained in 0.2 microgram of the gramicidin master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(27) *Vancomycin.* The term "microgram" applied to vancomycin means the vancomycin base activity (potency) contained in 1.25 micrograms of the vancomycin master standard.

(28) *Viomycin.* The term "microgram" applied to viomycin means the viomycin base activity (potency) contained in 1.355 micrograms of the viomycin master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(29) *Ampicillin.* The term "microgram" applied to ampicillin means the ampicillin activity (potency) contained in 1.1764 micrograms of the ampicillin master standard.

(30) *Nafcillin.* The term "microgram" applied to nafcillin means the nafcillin activity (potency) contained in 1.0989 micrograms of the nafcillin master standard.

(31) *Gentamicin.* The term "microgram" applied to gentamicin means the gentamicin activity (potency) contained in 1.56 micrograms of the gentamicin master standard when dried for 3 hours at 110° C. and a pressure of 5 millimeters or less.

(32) *Dactinomycin.* The term "microgram" applied to dactinomycin means the dactinomycin activity (potency) contained in 1,000 microgram of the dactinomycin master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(33) *Candicidin.* The term "microgram" applied to candicidin means the candicidin activity (potency) contained in 1.0 microgram of the candicidin master standard when dried for 3 hours at 40° C. and a pressure of 5 millimeters or less.

(34) *Cephalothin.* The term "microgram" applied to cephalothin means the cephalothin activity (potency) contained in 1.066 micrograms of the cephalothin master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(35) *Lincomycin.* The term "microgram" applied to lincomycin means the lincomycin base activity (potency) contained in 1.156 micrograms of the lincomycin master standard.

(36) *Cloxacillin.* The term "microgram" applied to cloxacillin means the cloxacillin activity (potency) contained in 1.135 micrograms of the cloxacillin master standard.

(37) *Methacycline.* The term "microgram" applied to methacycline means the methacycline activity (potency) contained in 1.082 micrograms of the methacycline master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(38) *Doxycycline.* The term "microgram" applied to doxycycline means the doxycycline activity (potency) contained in 1.155 micrograms of the doxycycline master standard.

(39) *Cephaloridine.* The term "microgram" applied to cephaloridine means the cephaloridine activity (potency) contained in 1.00806 micrograms of the cephaloridine master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(40) *Dicloxacillin.* The term "microgram" applied to dicloxacillin means the dicloxacillin activity (potency) contained in 1.087 micrograms of the dicloxacillin master standard.

(41) *Mithramycin.* The term "microgram" applied to mithramycin means the mithramycin activity (potency) contained in 1.000 microgram of the mithramycin master standard when dried for 4 hours at 25° C. and a pressure of 5 millimeters or less.

(42) *Clindamycin.* The term "microgram" applied to clindamycin means the clindamycin activity (potency) contained in 1.139 micrograms of the clindamycin master standard.

(43) *Cephaloglycin.* The term "microgram" applied to cephaloglycin means the cephaloglycin activity (potency) contained in 1.02564 micrograms of the cephaloglycin master standard.

(44) *Carbenicillin.* The term "microgram" applied to carbenicillin means the carbenicillin activity (potency) contained in 1.135 micrograms of the carbenicillin master standard.

(45) *Cephalexin.* The term "microgram" applied to cephalexin means the cephalexin activity (potency) contained in 1.0707 micrograms of the cephalexin master standard.

(46) *Penicillamine.* The term "microgram" applied to penicillamine means the amount of penicillamine in 1.000 microgram of the penicillamine master standard. The master standard has a purity of 100 percent.

(47) *Capreomycin.* The term "microgram" applied to capreomycin means the capreomycin activity (potency) contained in 1.0870 micrograms of the capreomycin master standard when dried for 4 hours at 100° C. and a pressure of 5 millimeters or less.

(48) *Rifampin.* The term "microgram" applied to rifampin means the rifampin activity (potency) contained in 1.0101 micrograms of the rifampin master standard.

(49) *Minocycline.* The term "microgram" applied to minocycline means the minocycline activity (potency) contained in 1.1588 micrograms of the minocycline master standard.

(50) *Spectinomycin.* The term "microgram" applied to spectinomycin means the spectinomycin activity (potency) contained in 1.490 micrograms of the spectinomycin master standard.

(51) *Clindamycin palmitate hydrochloride.* The term "microgram" applied to clindamycin palmitate hydrochloride means the clindamycin activity (potency) contained in 1.661 micrograms of the clindamycin palmitate hydrochloride master standard.

(52) *Carbenicillin indanyl.* The term "microgram" applied to carbenicillin indanyl means the carbenicillin activity (potency) contained in 1.4514 micrograms of the carbenicillin indanyl master standard.

(53) *Cephapirin.* The term "microgram" applied to cephapirin means the cephapirin activity (potency) contained in 1.0616 micrograms of the cephapirin master standard.

(54) *Cefazolin.* The term "microgram" applied to cefazolin means the cefazolin activity (potency) contained in 1.005 micrograms of the cefazolin master standard.

(55) *Mitomycin.* The term "microgram" applied to mitomycin means the mitomycin activity (potency) contained in 1.0416 micrograms of the mitomycin master standard.

Subpart B—Procedures for Antibiotic Drugs Affected by Drug Amendments of 1962

§ 430.10 Antibiotic drugs not subject to certification prior to May 1, 1963; statement of policy.

(a) Prior to the enactment of the 1962 amendments to the Federal Food, Drug, and Cosmetic Act, the only antibiotic drugs required to be submitted to the Food and Drug Administration for certification were those containing penicillin, streptomycin, chlortetracycline, chloramphenicol, or bacitracin, or any derivative of one of these antibiotics. Scientific proof of safety and efficacy was required. In the case of a drug containing any other antibiotic, it was necessary, unless the drug was then considered to be generally recognized as safe, that the applicant submit proof of safety under the new-drug provisions of the act. Furthermore, prior to enactment of the 1962 amendments, a number of these drugs had been declared no longer new drugs, since they had become generally recognized as being safe for their intended uses. As a result, there are now on the market antibiotic-containing drugs with labeling claims that, in the opinion of the Administration, are not supported by available medical data. This is particularly true for drugs such as troches, nose drops, mouth washes, and deodorants intended for use by the laity.

(b) Antibiotic drugs not subject to certification prior to May 1, 1963, therefore fall into two categories:

(1) Drugs for which, prior to their being marketed, the manufacturers ap-

plied for and obtained effective new-drug applications under the provisions of section 505 of the act.

(2) Drugs not cleared through the new-drug procedures prior to their being marketed.

(c) Under the provisions of the 1962 amendments, drugs described in paragraph (b)(1) of this section are exempt from an affirmative finding of efficacy for the conditions covered by the prior approval of the new-drug application, and the initial regulations listing them for certification for such conditions may not be withdrawn for lack of proof of efficacy until October 9, 1964. The deferred effective date as to efficacy does not apply to the drugs described in paragraph (b)(2) of this section.

(1) The Food and Drug Administration is now drafting regulations to provide for the certification of the drugs in paragraph (b)(1) of this section. Samples will be accepted from any manufacturer or repacker of a drug described in such regulations with a view to certification if the drug meets the requirements of the regulations or, pending the effective date of such regulations, with a view to release as provided in section 507(a) of the act. Thus, each manufacturer or repacker of a drug described in paragraph (b)(1) of this section need not hold an effective new-drug application for the drug in order to qualify for certification, but all such drugs will be certified only with the claims for those conditions for which the drugs were found to be safe under the new-drug procedures. If a regulation providing for the certification of a drug in paragraph (b)(1) has not been revoked before October 9, 1964, it will be revoked after that date unless the Commissioner of Food and Drugs has received substantial evidence to support the claims of effectiveness.

(2) The Commissioner does not intend to issue regulations to provide for the certification of any drug covered by paragraph (b)(2) of this section until he has received substantial evidence to support such regulations. A drug in this group, even though marketed before October 10, 1962, may not now be marketed until an appropriate regulation has been issued and the drug has been certified or the drug has been released as provided in section 507(a) of the act. To permit orderly transition to the certification requirements, the Commissioner will continue to accept samples of a drug in this group with a view to release of batches as provided in section 507(a) of the act, provided the manufacturer submits to the Food and Drug Administration by September 6, 1963, evidence to support the claims for such drugs.

§ 430.11 Certification, release, or exemption of antibiotic-containing drugs.

(a) There was published in the FEDERAL REGISTER of July 23, 1963 (28 FR 7473), under § 146.31 (now § 430.10) of the general regulations for the certification of antibiotic and antibiotic-containing drugs a statement of policy providing for among other things, an "Orderly transition to the certification requirements" by making eligible for release on an interim basis batches of antibiotic drugs previously marketed without new-drug approvals and not subject to certification prior to May 1, 1963. There were published in the FEDERAL REGISTER of March 9, 1966 antibiotic drug regulations providing for certification of certain drugs brought under the certification requirements of section 507 of the act by the Kefauver-Harris Drug Amendments of 1962. These are antibiotic-containing drugs for which new-drug approvals are in effect solely on the evidence of their safety. Regulations have also been published to provide for certification of all antibiotic drugs for which adequate evidence of safety and efficacy has been submitted under applicable provisions of the act and the regulations.

(b) The Commissioner finds that persons marketing antibiotic-containing drugs prior to enactment on October 10, 1962, of the Kefauver-Harris Drug Amendments have been afforded ample time to submit evidence of the safety and efficacy of such drugs.

(c) In view of the foregoing, the Commissioner has determined that no preparation containing antibiotic drugs and intended for administration to man will be certified, released, or exempted from certification, except as provided in paragraph (d) of this section.

(d) (1) In the case of antibiotic-containing drugs subject to certification solely on the evidence of safety contained in approved new-drug applications, pursuant to requirements of section 507(h) of the act, certification or release of such articles will continue pending the repeal of such regulations.

(2) Any antibiotic-containing drug required to be sold on prescription, for which the manufacturer has submitted additional data and has received no adverse evaluation of such data from the Food and Drug Administration, will continue to be eligible for release until the data has been evaluated and the manufacturer notified. Since this review is expected to be accomplished by September 1, 1966, the manufacturer of such drugs should submit any additional information promptly, preferably in the form of an application for certification of such drug as prescribed by Part 431 of this chapter. This provision shall not apply to any drug for which the Commissioner makes a determination that such drug is unsafe or where the Commissioner concludes that such drug is not efficacious.

Subpart C—Administrative Procedures

§ 430.20 Procedure for the issuance, amendment, or repeal of regulations.

(a) The procedures for the issuance, amendment, or repeal of regulations under section 507 of the act are designed to permit swift action whenever the factual situation makes such action necessary or desirable. Ordinarily, such regulations are developed by the Food and Drug Administration with the consultation and advice of the interested persons. This permits the publication of regulations that become effective on the date of publication when they are in the public interest and present no significant points of controversy. Regulations that become effective immediately may also be published when the Commissioner finds they are necessary to deal with an imminent hazard to the public health. Proposed regulations are published with opportunity for written comment and informal conferences whenever such proposals have not been made the subject of prior consultation with and the agreement of interested persons. Section 507(f) and the review provisions of section 701(f) and (g) of the act are applicable (1) to proposals to amend or repeal regulations providing for certification or exemption from certification of antibiotic drugs subject to the provisions of section 507(h) on the grounds of lack of substantial evidence of effectiveness and (2) as needed to insure the right of protest and a public hearing to all interested persons who have reasonable grounds for dissatisfaction with the Food and Drug Administration's action with respect to regulations.

(b) An order issuing, amending, or repealing any regulation contemplated by section 507 of the act may be made effective on the date of its publication in the FEDERAL REGISTER whenever the Commissioner finds that:

(1) Such an order has been prepared in consultation with the interested persons, is in the public interest, and presents no significant points of controversy; or

(2) Such an order is necessary to deal with an imminent hazard to the public health.

(c) Such regulations may be initiated by any interested person on the basis of applications, request, or data submitted, including for example a request to provide for certification of a new antibiotic product (§ 431.50 of this chapter, form 5). Such regulations may be initiated by the Commissioner on the basis of information such as records and reports submitted under § 431.60 of this chapter or reports from the National Academy of Sciences—National Research Council, Drug Efficacy Study Group, or other information. An order issued under the provisions of paragraph (b)(2) of this section shall furnish interested persons who are adversely affected by such order opportunity to file objections to it, within 30 days after its publication, specifying with particularity the changes desired and stating reasonable grounds therefor, and to request a public hearing upon the objections. The filing of such objections and requests shall not stay the order, but the Commissioner shall thereupon, after due notice, provide for such public hearing. In such cases, the provisions of Subpart F of Part 2 of this chapter shall apply to such hearings, except as modified by paragraph (f) of this section, and to judicial review in accord with section 701 (f) and (g) of the act.

(d) (1) The Commissioner, on his own initiative or on the application or request of any interested person, may publish in

the FEDERAL REGISTER a notice of proposed rule making and order to issue, amend, or repeal any regulation contemplated by section 507 of the act. Such notice and order may be general (i.e., simply summarizing in a general way the information resulting in the notice and order) or specific (i.e., either referring to specific requirements in the statute and regulations with which there is a lack of compliance, or providing a detailed description and analysis of the specific facts resulting in the notice and order).

(2) An opportunity shall be given for interested persons to submit written comments and to request an informal conference on the proposal, unless such notice and opportunity for comment and informal conference have already been provided in connection with the announcement of the reports of the National Academy of Sciences-National Research Council, Drug Efficacy Study Group, to persons who will be adversely affected, or unless the no-controversy or imminent hazard conditions set forth in paragraph (b) of this section have been met. The time for requesting an informal conference shall be 30 days and the time for comment shall be 60 days unless otherwise specified in the notice of proposed rule making. If an informal conference is requested and granted, those persons participating in the conference shall be provided an additional 30 days for comment, beginning the date of the conference, unless otherwise specified in the proposal.

(3) It is the responsibility of every manufacturer or distributor of an antibiotic drug product to review every proposal published in the FEDERAL REGISTER to determine whether it covers any product he manufactures or distributes.

(4) After considering the written comments, the results of any conference, and the data available, the Commissioner will publish an order in the FEDERAL REGISTER acting on the proposal, with opportunity for any person who will be adversely affected to file objections, to request a hearing, and to show reasonable grounds for the hearing. Any such person who elects to avail himself of the opportunity for a hearing shall file (i) within 30 days after the date of publication of the order a written notice of appearance and request for hearing, and (ii) within 60 days after the date of publication of the order, unless a different period of time is specified in the order, the studies on which he relies to justify a hearing as specified in paragraph (d)(8) of this section.

(5) All data and information (including any protocols and all underlying raw data) shall be included in full and may not be incorporated by reference, except that raw data underlying a study submitted may be incorporated by reference from a prior submission as part of an antibiotic application, or other applications or reports. A copy of any article cited shall be included. If any part of the submission is in a foreign language, an accurate and complete English translation shall be appended to such part. Translations of literature printed in a foreign language shall be accompanied by the original publication.

(6) All submissions shall be made in quintuplicate and filed with the Hearing Clerk, Food and Drug Administration, Room 6–86, 5600 Fishers Lane, Rockville, MD 20852.

(7) No data or analysis submitted after such 60 days will be considered in determining whether a hearing is warranted unless they are derived from well-controlled studies begun prior to the date of the order, the results of which were not in existence during that 60 days. Exceptions may be made on the basis of a showing of inadvertent omission and hardship. All studies in progress, the results of which the person requesting the hearing intends later to submit in support of the request for hearing, shall be listed. A copy of the complete protocol, a list of the participating investigators, and a brief status report of the studies shall be included in the submission made pursuant to paragraph (d)(4)(ii) of this section.

(8) A request for hearing shall be supported by a submission as specified in § 314.200(c)(1)(ii) of this chapter containing the studies (including all underlying raw data) on which the person relies to justify a hearing with respect to his drug product.

(i) If effectiveness is at issue, a request for hearing shall be supported only by adequate and well-controlled clinical studies meeting all of the precise requirements of § 314.111(a)(5) of this chapter and, for combination drug products, § 3.86 of this chapter, or by other studies not meeting those requirements for which a waiver has been previously granted by the Food and Drug Administration pursuant to the provisions of § 314.111(a)(5) of this chapter. All adequate and well-controlled clinical studies on the drug product known to the person requesting the hearing shall be submitted. Any unfavorable analyses, views, or judgments with respect to such studies known to such person shall also be submitted. No other data, information, or studies shall be submitted.

(ii) Such submission shall include a factual analysis of all studies submitted. If effectiveness is at issue, such analysis shall specify how each such study accords, on a point-by-point basis, with each criterion required for an adequate and well-controlled clinical investigation established in § 314.111(a)(5) of this chapter and, if the product is a combination drug product, with each of the requirements for a combination drug established in § 3.86 of this chapter, or shall be accompanied by an appropriate waiver previously granted by the Food and Drug Administration. If a study deals with a drug entity or dosage form, or condition of use, or mode of administration other than the one(s) in question, such fact(s) shall be clearly stated. Any study conducted on the final marketed form of the drug product shall be so designated.

(iii) Such analysis shall be submitted in the following format, except that information relating to safety or effectiveness shall be omitted if the order does not raise any issue with respect to that aspect of the drug; and information on compliance with § 3.86 of this chapter shall be omitted if the drug product is not a combination drug product. Submissions not made in this format or not containing the required analyses will not be considered and will result in denial of hearing, except that minor technical deficiencies may be excused if it is apparent that a good faith attempt has been made to comply with the requirements of this section and any deficiencies noted are immediately corrected upon request.

I. Safety data.

A. Animal safety data.

1. Individual active component(s).

a. Controlled studies.

b. Partially controlled or uncontrolled studies.

2. Combinations of the individual active components.

a. Controlled studies.

b. Partially controlled or uncontrolled studies.

B. Human safety data.

1. Individual active component(s).

a. Controlled studies.

b. Partially controlled or uncontrolled studies.

c. Documented case reports.

d. Pertinent marketing experiences that may influence a determination as to the safety of each individual active component.

2. Combinations of the individual active components.

a. Controlled studies.

b. Partially controlled or uncontrolled studies.

c. Documented case reports.

d. Pertinent marketing experiences that may influence a determination as to the safety of combinations of the individual active components.

II. Effectiveness data.

A. Individual active components: Controlled studies, with an analysis showing clearly how each such study satisfies, on a point-by-point basis, each of the criteria required by § 314.111(a)(5) of this chapter.

B. Combinations of individual active components.

1. Controlled studies with an analysis showing clearly how each such study satisfies, on a point-by-point basis, each of the criteria required by § 314.111(a)(5) of this chapter.

2. An analysis showing clearly how each requirement of § 3.86 of this chapter has been satisfied.

III. A summary of the data and views setting forth the medical rationale and purpose for the drug and its ingredients and the scientific basis for the conclusion that the drug and its ingredients have been proven safe and/or effective for the intended use. If there is an absence of controlled studies in the material submitted, or the requirements of any element of § 3.86 of this chapter or § 314.111(a)(5) of this chapter have not been fully met, such fact(s) shall be clearly stated, and a waiver obtained pursuant to § 314.111(a)(5) of this chapter shall be enclosed.

IV. A statement signed by the person responsible for such submission, that it includes in full (or incorporates by reference as permitted in § 430.20(d)(1)) all studies and information specified in § 430.20(d). (Warning: A willfully false statement is a criminal offense, 18 U.S.C. 1001.)

(9) Upon receipt of any request for hearing, the Director of the Bureau of Drugs shall prepare an analysis of the

request and a proposed order ruling upon the matter. The analysis and proposed order, the request for hearing, and any proposed order denying a hearing and response pursuant to paragraph (d)(10)(ii) or (iii) of this section, shall be submitted to the office of the Commissioner for independent review and decision. No representative of the Bureau of Drugs shall participate or advise in the review and decision by the Commissioner. The office of the General Counsel shall observe the same separation of functions.

(10) A request for a hearing may not rest upon mere allegations or denials, but must set forth specific facts showing that there is a genuine and substantial issue of fact with respect to the particular drug product(s) which is specified in the request for hearing that requires a hearing.

(i) Where a specific proposal or order (as defined in paragraph (d)(1) of this section) is used, the order published in the FEDERAL REGISTER shall state that, if it conclusively appears from the face of the data, information, and factual analyses in the request for hearing that there is no genuine and substantial issue of fact which precludes the action taken on the proposal, e.g., no adequate and well-controlled clinical investigations meeting each of the precise elements of § 314.111(a)(5) of this chapter and, for a combination drug product, § 3.86 of this chapter, showing effectiveness have been identified, or when a request for hearing is not made in the required format or with the required analyses, the Commissioner will enter summary judgment against the person(s) who requests a hearing, making findings and conclusions, denying a hearing. Any such order entering summary judgment shall set forth the Commissioner's findings and conclusions in detail and shall specify why each study submitted fails to meet the requirements of the statute and regulations or why the request for hearing otherwise does not raise a genuine and substantial issue of fact or shall specify the requirements of this paragraph with respect to format or analyses with which there is a lack of compliance.

(ii) Where a general notice or order (as defined in paragraph (d)(1) of this section) is used and the Director of the Bureau of Drugs concludes that summary judgment against the person(s) requesting a hearing should be considered, he shall serve upon such person(s) by registered mail a proposed order denying a hearing. Such person(s) shall have 60 days after receipt of such proposed order to respond with sufficient data, information and analyses to demonstrate that there is a genuine and substantial issue of fact which justifies a hearing.

(iii) Where a general or specific notice or order is used and the person(s) requesting a hearing submits data or information of a type required by the statute and regulations, and the Director of the Bureau of Drugs concludes that summary judgment against such person(s) should be considered, he shall serve upon such person(s) by registered mail a proposed order denying a hearing. Such person(s) shall have 60 days after receipt of such proposed order to respond with sufficient data, information, and analyses to demonstrate that there is a genuine and substantial issue of fact which justifies a hearing.

(iv) If review of the data, information, and analyses submitted warrants the conclusion that the basis for the order is not valid, e.g., that substantial evidence of effectiveness exists, the Commissioner shall deny the hearing, enter summary judgment for the person(s) requesting the hearing, and revoke the order. If a hearing is not requested, the order will become effective as published.

(v) If a hearing is requested and justified, the Commissioner will issue a written notice defining the issues, naming an administrative law judge, and specifying the time and place at which the hearing will commence, which shall be as soon as practicable. The provisions of Subpart F of Part 2 of this chapter shall apply to such hearing, except as modified by paragraph (f) of this section.

(vi) A hearing shall be granted if there exists a genuine and substantial issue of fact or if the Commissioner concludes, in his discretion, that a hearing would otherwise be in the public interest.

(11) Any hearing will be open to the public except that any portion of the hearing concerning a method or process that the Commissioner finds is entitled to protection as a trade secret pursuant to section 301(j) of the act (21 U.S.C. 331(j)) or 18 U.S.C. 1905 will not be open to the public unless the respondent specifies otherwise in his appearance. All persons who have requested a hearing and for whom a hearing has been granted shall be parties to the hearing. Interested persons who are not parties may appear at and participate in a hearing and shall have the right to present evidence and file pleadings relevant to the issues. Such interested persons may otherwise participate, e.g., cross-examine witnesses, when in the judgment of the administrative law judge their interests are not adequately protected otherwise or it is required for a full and true disclosure of the facts.

(12) The repeal of any regulation constitutes a revocation of all outstanding certificates based upon such regulation. However, the Commissioner may, in his discretion, defer or stay such action pending a ruling on any related request for a hearing or pending any related hearing or other administrative or judicial proceeding.

Effective date. § 430.20(d) was effective on April 12, 1974. All submissions to the Food and Drug Administration on or after that date must be in compliance with it. No request for hearing submitted prior to the effective date of § 430.20(d) may be supplemented subsequent to such effective date except for studies already begun as of that date.

(e) Whenever any interested person submits an application or request pursuant to provisions of section 507 of the act, or regulations promulgated thereunder, which application or request contemplates the issuance, amendment, or repeal of any regulation, and such person has been informed in writing that such application or request is not approvable, or whenever such person has received no written communication advising whether or not such application is approvable by the 180th day after its submission, such interested person may file a petition proposing the issuance, amendment, or repeal of such regulation under the provision of section 507(f) of the act and Subpart F of Part 2 of this chapter. The Commissioner shall cause the regulation proposed in such petition to be published in the FEDERAL REGISTER within 60 days of the receipt of an acceptable petition and further proceedings shall be in accord with the provisions of sections 507(f) and 701 (f) and (g) of the act and Subpart F of Part 2 of this chapter except as modified by paragraph (f) of this section.

(f) The provisions of Subpart F of Part 2 of this chapter shall be applicable to proceedings under the provisions of section 507(f) of the act as therein set forth with the following modification: The provisions of § 2.63(a) of this chapter notwithstanding, at any hearing held pursuant to section 507(f) of the act which involves a question of the safety or effectiveness of an antibiotic drug, the burden of proof of safety and substantial evidence of effectiveness under the questioned conditions shall be on the parties contending that the drug is safe and effective for the conditions for which it is prescribed, recommended or suggested in the labeling thereof.

(g)(1) No regulation providing for the certification of any batch of any drug composed wholly or in part of any kind of penicillin, streptomycin, chlortetracycline, chloramphenicol, bacitracin, or any other antibiotic drug, or any derivative thereof, intended for use by man shall be promulgated and no existing regulation will be continued in effect unless it is established by substantial evidence that the drug will have such characteristics of identity, strength, quality, and purity necessary to adequately insure safety and efficacy of use. "Substantial evidence" has been defined by Congress to mean "evidence consisting of adequate and well-controlled investigations, including clinical investigations, by experts qualified by scientific training and experience to evaluate the effectiveness of the drug involved, on the basis of which it could fairly and responsibly be concluded by such experts that the drug will have the effectiveness it purports and is represented to have under the conditions prescribed, recommended or suggested in the labeling thereof." This definition is made applicable to a number of antibiotic drugs by section 507(h) of the act. And it is the test of efficacy that will be applied in promulgating, amending, or repealing regulations for the certification of all antibiotics under section 507(a) of the act as well.

(2) The scientific essentials of an adequate and well-controlled clinical investigation are described in § 314.111(a)(5) of this chapter.

PART 431—CERTIFICATION OF ANTIBIOTIC DRUGS

Subpart A—General Provisions

Sec.
431.1 Requests for certification, check tests and assays, and working standards; information and samples required.
431.5 Samples for sterility testing.
431.10 Certification.
431.11 Conditions on the effectiveness of certificates.
431.12 Certification of antibiotic drugs after shipment in bulk containers.
431.16 Changes in facilities or controls; changes in mailing or promotional pieces.
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Subpart B—Administrative Procedures

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431.51 Suspension of certification service.
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Subpart C—Records and Reports

431.60 Records and reports concerning experience with antibiotic drugs for human use for which a certificate or release has been issued.
431.61 Records of distribution.
431.62 Records retention.

AUTHORITY: Sec. 507, 59 Stat. 463, as amended (21 U.S.C. 357), unless otherwise noted.

Subpart A—General Provisions

§ 431.1 Requests for certification, check tests and assays, and working standards; information and samples required.

(a) A request for certification of a batch shall be addressed to the Commissioner and shall be in a form specified by him. A request from a foreign manufacturer shall be signed by such manufacturer and by an agent of such manufacturer who resides in the United States.

(b) (1) The initial request for certification of a batch of any drug submitted by any person shall be preceded or accompanied by a full statement of the facilities and controls used to maintain the identity, strength, quality, and purity of each batch of such drug, including descriptions of:

(i) The methods and processes used in the manufacture of the drug;

(ii) The tests and assays of the drug made during the manufacture of the batch and after it is packaged; and

(iii) The laboratory facilities used in such controls.

(2) Such initial request shall also be preceded or accompanied by the key of the batch marks used by such person and by specimens of all labeling to be used for such drug.

(c) A person who requests certification or check tests and assays of a batch shall submit with his request the following information and samples:

(1) The batch mark of the drug.

(2) The quantity of each ingredient used in making the batch and a statement that each such ingredient conforms to the requirements or standards prescribed therefor, if any, by specific regulations or official compendium or otherwise approved by the Commissioner.

(3) The size of the batch, including the number of containers of each size in the batch.

(4) The date of the latest assay of the batch.

(5) The results of the latest tests and assays made by or for him on the batch as required for the drug by specific regulations.

(6) The batch mark(s) of the antibiotic(s) used in making the batch.

(7) Unless previously submitted, the results and dates of the latest tests and assays made by or for him on the antibiotic(s) used in making the batch as required by specific regulations.

(8) The number of accurately representative samples that are required for the batch by specific regulations:

(i) In the case of drugs such as dry powders, solutions, ointments, and suspensions, the sample shall be collected by taking single immediate containers, before or after labeling, at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal. In no case, however, shall more than 5,000 immediate containers have been packaged during each such interval of sampling, except for a sample collected for sterility testing.

(ii) In the case of drugs such as tablets or other such unit dosage forms, the sample shall be collected by taking single tablets at such intervals throughout the entire time of tableting the batch that the quantities tableted during the intervals are approximately equal. In no case, however, shall more than 5,000 tablets have been tableted during each interval of sampling, except for a sample collected for time of distintegration. If the person who packages the tablets into dispensing-size containers is not the manufacturer, such sample shall be collected throughout the entire time of packaging the batch into such containers.

(iii) In the case of drugs packaged for repacking or for use in the manufacture of another drug, the sample must be representative of the batch. Such samples may be taken from a composite composed of portions taken from a representative number of bulk containers, the composite consisting of no more than 10 times the amount required for conducting the required tests and assays. Such samples are not required if they have been previously submitted.

(iv) In the case of a sterile drug packaged in combination with containers of a sterile diluent, the sample shall be collected by taking 20 immediate containers of the diluent collected at regular intervals throughout each filling operation, except that if the diluent is sterilized after filling into containers, the representative sample shall consist of 20 immediate containers collected from each sterilizer load and each container shall be taken from a different part of each such sterilizer load. In the case of sterile drugs packaged in combination with sterile droppers, the sample shall be collected by taking 20 droppers from each sterilizer load and each stopper shall be taken from a different part of such sterilizer load.

(9) In the case of an initial request for certification, each ingredient used in making the batch other than ingredients required by specific regulations: 1 package of each containing approximately 5 grams. Results and dates of the latest tests and assays made by or for him on such ingredients shall precede or accompany the submission.

(10) The results and dates of tests and assays made by or for him on the nonantibiotic active ingredients in the batch.

(11) If such batch or any part thereof is to be packaged with a sterile diluent or sterile dropper, such request shall also be accompanied by a statement that such diluent or dropper is sterile and conforms to the requirements prescribed therefor by specific regulations.

(d) Each sample submitted pursuant to the regulations in this chapter shall be addressed to the Commissioner. Its package shall be clearly identified as to its contents and shall bear the name and post-office address of the person submitting it.

(e) In addition to the information and samples specifically required to be submitted to the Commissioner by the regulations in this chapter, the person who requests certification of a batch shall submit such further information and samples as the Commissioner may require for the purpose of investigations to determine whether or not such batch complies with the requirements of § 431.10 for the issuance of a certificate.

(f) Upon the request of any person, stating reasonable grounds therefor, the Commissioner shall furnish such person with a portion of the working standards.

§ 431.5 Samples for sterility testing.

(a) ***"Filling operation" and "sample" defined.*** (1) The term "filling operation" when used in connection with samples of a batch required for sterility testing refers to that period of time not longer than 24 consecutive hours during which a homogeneous quantity of a drug is being filled continuously into market-size containers and during which no changes are made in the equipment used for filling. (Short rest periods for operators of the filling equipment and the time required to change operators between consecutive shifts are not considered as a break in continuity of the filling operation.) If more than one filling device is used during the filling operation, the samples shall include immediate containers filled by each device, and each such container shall be identified with a mark corresponding to that assigned to the filling device. If more than one filling operation is required to fill a batch, each container in the sample shall be identified with the number of the operation.

(2) For the purpose of sterility testing, the term "sample" means the total number of containers taken from each filling operation.

(b) *Packaging requirements for samples.* If a batch of a sterile antibiotic is packaged for repacking or for use as an ingredient in the manufac-

ture of another drug, the sample required for sterility testing may be packaged in one container, in lieu of 20 containers, or in two containers in lieu of 40 containers, under the following conditions:

(1) The weight or volume of the sample is equivalent to the composite weight or volume required for a multiple container sample;

(2) The sample is a composite of samples taken from all parts of the batch; and

(3) The sterility test method prescribed for the drug by the regulations in this chapter is "Bacterial membrane filter method" described in § 436.20(e)(1) of this chapter.

§ 431.10 Certification.

(a) If it appears to the Commissioner, after such investigation as he considers necessary, that:

(1) The information (including results of tests and assays) and samples required by or pursuant to the regulations in this chapter have been submitted, and the request for certification contains no untrue statement of a material fact; and

(2) The batch complies with the regulations in this chapter and conforms to the applicable standards of identity, strength, quality, and purity prescribed by the regulations in this chapter;

the Commissioner shall certify that such batch is safe and efficacious for use, subject to such conditions on the effectiveness of certificates as are prescribed by § 431.11 and shall issue to the person who requested it a certificate to that effect.

(b) If the Commissioner determines, after such investigation as he considers to be necessary, that the information submitted pursuant to the regulations in this chapter, or the batch covered by such request, does not comply with the requirements set forth in paragraph (a) of this section for the issuance of a certificate, the Commissioner shall refuse to certify such batch and shall give notice thereof to the person who requested certification, stating his reasons for refusal.

(c) All statements, samples, and other information and materials submitted in connection with a request for certification shall be considered to be part of such request.

(d) Compliance of a drug with the standards of identity, strength, quality, and purity prescribed by regulations in this chapter shall be determined by the tests and methods of assay prescribed for such drug by regulations issued under this chapter.

(e) The regulations in this chapter, prescribing tests and methods of assay for antibiotic and antibiotic-containing drugs, shall not be construed as preventing the Commissioner from using any other test or method of assay in his investigations to determine whether or not:

(1) A request for certification contains any untrue statement of a material fact; or

(2) A certification has been obtained through fraud, or through misrepresentation or concealment of a material fact.

(f) Except as specifically provided by the regulations in this chapter, no provision of any regulation shall be construed as exempting any certifiable antibiotic drug from any applicable provision of the act or any regulation thereunder.

§ 431.11 Conditions on the effectiveness of certificates.

(a) A certificate shall not become effective:

(1) If it is obtained through fraud or through misrepresentation or concealment of a material fact;

(2) With respect to any package unless it complies with the packaging requirements, if any, prescribed by the regulations in this chapter which were in effect on the date of the certificate;

(3) With respect to any package unless its label and labeling bear all words, statements, and other information required by the regulations in this chapter; or

(4) With respect to any package of a certifiable antibiotic drug subject to the regulations in this chapter, when it is included in a packaged combination with another drug, unless such other drug complies with the requirements of the regulations in this chapter.

(b) A certificate shall cease to be effective:

(1) With respect to any immediate container after the expiration date, if any, prescribed by the regulations in this chapter;

(2) With respect to any immediate container when it or its seal (if the regulations in this chapter require it to be sealed) is broken, or when its label or labeling is altered, mutilated, destroyed, obliterated, or removed in whole or in part, or ceases to conform to any labeling requirement prescribed by the regulations in this chapter, except that:

(i) If the drug in such container is repacked or used as an ingredient in the manufacture of another drug, and certification of the batch thus made is requested, such certificate shall continue to be effective for a reasonable time to permit certification or destruction of such batch;

(ii) If the drug is in a container packaged for dispensing and is used in compounding a prescription issued by a practitioner licensed by law to administer such drug, such certificate shall continue to be effective for a reasonable time to permit the delivery of the drug compounded on such prescription; or

(iii) If its label or labeling is removed in whole or in part for the purpose of relabeling and supplemental certification of the relabeled drug is requested, as provided by § 433.12 of this chapter.

(3) With respect to any immediate container of penicillin when it is included in the packaged combination penicillin with aluminum hydroxide gel or penicillin with a vasoconstrictor, or to any immediate container of bacitracin when it is included in the packaged combination bacitracin with a vasoconstrictor, except that when certification of the batch so included is requested, such certificate shall continue to be effective for a reasonable time to permit certification of such batch which is part of such combination;

(4) With respect to any package when the drug therein fails to meet the standards of identity, strength, quality, and purity which were in effect on the date of the certificate; except that those minor changes which occur before the expiration date and which are normal and unavoidable in good storage and distribution practice shall be disregarded.

(5) With respect to any package of a certifiable antibiotic drug subject to the regulations in this chapter, included in a packaged combination with another drug, when such other drug fails to meet the requirements of the regulations in this chapter; or

(6) With respect to any immediate container, if such regulations require its labeling to bear a caution against dispensing otherwise than on prescription, at the beginning of the act of dispensing or offering to dispense it otherwise than:

(i) By a practitioner licensed by law to administer such drug; or

(ii) On his prescription issued in his professional practice.

§ 431.12 Certification of antibiotic drugs after shipment in bulk containers.

(a) The Food and Drug Administration has received inquiries from certain interested manufacturers concerning their shipment of certified antibiotics, packaged in bulk containers, to hospitals and pharmacies for repacking or for use in the manufacture of another drug on the order or prescription of a physician. The regulations promulgated under section 507 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 357) do not prohibit the shipment of certified bulk containers of antibiotics to such persons. However, under the provisions of § 431.11(b)(2)(i), certification should be requested of each repacked batch and of each batch of another drug manufactured from such bulk drug, unless the repackaged drug or other drug has been made exempt from the certification requirements by regulation. The fact that the drug is to be repacked or manufactured on the order or prescription of a physician does not exempt it from the certification requirements of the act. Under the provisions of § 431.11(b)(2)(ii), it is only when the drug used to compound a prescription is in a container packaged for dispensing that certification of the drug so compounded is not required.

(b) In the light of these provisions, unless the manufacturer and shipper of bulk containers of antibiotics has, with the consignee, an effective permit issued under § 433.16 of this chapter, if the drug is to be repacked, or under § 433.13 of this chapter if it is to be used in the manufacture of another drug, the shipper has the responsibility of seeing that certification is requested of each repacked batch and of each batch of another drug manufactured from such drug.

§ 431.16 Changes in facilities or controls; changes in mailing or promotional pieces.

(a) Before a person makes a change in the facilities or controls used in the manufacture, packaging, or labeling of a drug described pursuant to § 431.1(b), he shall submit to the Commissioner for advance approval a full statement describing the proposed change. In the case of a proposal to use revised labeling on or within the drug package or promotional labeling containing information for use of the drug that is not the same in language and emphasis as the approved labeling, the applicant shall submit specimens for advance approval. Advance approval is not required when pursuant to a published FEDERAL REGISTER notice implementing a NAS–NRC drug efficacy study, the package labeling, other promotional labeling, or an advertisement is revised (i) to delete a claim for which there is a lack of substantial evidence of effectiveness, (ii) to modify labeling to be consistent with such notice, or (iii) to add in accord with § 3.81 of this chapter an informative statement of the findings of the National Academy of Sciences-National Research Council, Drug Efficacy Study Group, with respect to any claim in the labeling evaluated as other than "effective."

(b) In the case of mailing and promotional pieces that contain the same information for use of the drug as previously approved labeling, in which any other information is consistent with and not contrary to such labeling in accord with § 1.106(b) of this chapter and so certified by the applicant (or authorized representative), the applicant shall submit specimens when first used and need not await advance approval.

§ 431.17 New antibiotic and antibiotic-containing products.

Any request that the Secretary provide for the certification of batches of a drug for which no provision for certification is made in the existing regulations in this chapter shall be in a form specified by the Commissioner and shall be accompanied by:

(a) A statement of the conditions for which the person who makes such request intends such drug to be used, and adequate directions for use in each such condition;

(b) Full reports of investigations which have been made to show whether or not such drug is safe and efficacious for use in such conditions;

(c) A full list of the articles used as components of such drug;

(d) A full statement of the composition of such drug;

(e) A full description of the methods used in, and the facilities and controls used for, the manufacture, processing, and packaging of such drug;

(f) A full description of, or references to publications containing practical and accurate tests and methods of assay to determine the identity, strength, quality, and purity of such drug;

(g) Such samples of such drug and of the articles used as components thereof as the Commissioner may require; and

(h) Specimens of all labeling proposed to be used for such drug, including that to be used in its promotion.

(i) An environmental impact analysis report analyzing the environmental impact of the manufacturing process and the ultimate use or consumption of the antibiotic drug pursuant to § 6.1 of this chapter.

§ 431.20 Disposition of outdated drugs.

When certification becomes invalid because the expiration date is passed, such articles should not be disposed of for drug use either through commercial or charitable channels unless the articles have been assayed to establish potency and recertified.

Subpart B—Administrative Procedures

§ 431.50 Forms for certification or exemption of antibiotic drugs.

The following forms which must be supplied in connection with certain certification or exemption procedures for antibiotic drugs may be obtained from the Certification Services Staff (HFD–145), Food and Drug Administration, Department of Health, Education, and Welfare, 5600 Fishers Lane, Rockville, MD 20852.

Form	
1	Application for exemption for storage.
2	Application for exemption for processing.
3	Application for exemption for labeling.
4	Application for exemption for manufacturing use.
5	Request to provide for certification of a new antibiotic product (the form provided for in § 135.4 of this chapter is to be used if the antibiotic is intended to be used in animals).
6	Data to accompany or precede every initial request for certification of a batch of an antibiotic drug.
7	Request for check tests and assays or certification of a batch of _____ (the blank to be filled in with the name of the antibiotic drug).
8	Application for exemption for repacking.
9	Request for supplemental certification of a batch of an antibiotic drug.

§ 431.51 Suspension of certification service.

When the Commissioner finds that a person has:

(a) Obtained or attempted to obtain a certificate through fraud or through misrepresentation or concealment of a material fact; or

(b) Falsified the records required to be kept by § 431.61, or

(c) Failed to keep such records or to make them available, or to accord full opportunity to take an inventory of stocks on hand, or otherwise to check the correctness of such records as required by § 431.61; or

(d) Failed to establish a system for maintaining the records required by § 431.60 or has repeatedly or deliberately failed to maintain such records or to make required reports in accordance with the provisions of that section, or has refused to permit access to, or copying, or verification of such records or reports; or

(e) Failed to conform to the requirements of good manufacturing practice prescribed by Part 133 of this chapter; the Commissioner will immediately suspend service to such person under the regulations in this chapter. Upon request a hearing will be granted to such person to show cause why such service should be resumed.

§ 431.52 Hearing procedure.

Hearings held pursuant to § 431.51 will be conducted in accordance with the rules provided in Part 314 of this chapter.

§ 431.53 Fees.

(a) Fees for the services rendered under the regulations in this chapter shall be such as are necessary to provide, equip, and maintain an adequate certification service.

(b) The fee for such services with respect to each batch of a drug, certification of which is provided by the regulations in this chapter, including those published hereafter, is the sum of the fees for all tests required for certification of each batch. The minimum tests for each batch shall be those prescribed in the section relating specifically to such drug.

(1) The fee schedule for antibiotic drug certification is as follows:

Test:	*Chargeable fee per test*
Arquad content	$27
Butanol content	55
Candioidin potency (special turbidimetric)	55
Capreomycin I content	242
Color identity	10
Column chromatographic isomer content	109
Crystallinity	4
Cycloserine color assay	37
Dactinomycin potency (special plate)	55
Disc potency	31
Doxycycline purity (paper chromatography)	110
Free chloride	81
Gas chromatography	55
Gentamicin C	185
Heavy metals test	18
Histamine test	35
Infrared identity	23
Infrared quantitative	47
Iodochlorhydroxyquin content	27
Karl Fisher moisture	10
LD_{50} toxicity	216
Loss on drying	14
Melting range	9
Metal particles (ophthalmic ointments)	27
Microbiological assay, plate	25
Microbiological assay, turbidimetric	13
Micro-organism count	59
Minocycline content	109
Nonaqueous titrations (and complexiometric)	11
Paper chromatographic identity	55
Penicillin chemical assay	12
Penicillin contamination	27
Penicillin G content	27
pH	4
Probenecid content	34
Procaine colorimetric	10
Pyrogens test: 3 rabbits	115
Pyrogens test: 8 rabbits	229
Residual streptomycin	13
Residue on ignition	32
Safety test	24
Specific rotation	18

Test:	Chargeable fee per test
Specific surface area	27
Sterility test	59
Sulfate content	11
Tablet disintegration	6
Thin layer chromatographic identity	27
Total chlorine	81
Undecylenic acid content	23
Ultraviolet absorptivity	23
Ultraviolet identity	23
Ultraviolet potency	23
Vancomycin identity	162
Zinc titration	36

(2) In the case of a supplemental request submitted pursuant to the provisions of § 433.12 of this chapter, the fee shall be $8.

(3) In the case of persons using the certification services whose manufacturing facilities are not located in the United States or the Commonwealth of Puerto Rico, such persons shall be required to deposit each year sufficient funds to cover costs encountered when their facilities are inspected pursuant to the provisions of section 704 of the act.

(c) When the Commissioner considers it necessary to make investigations of a new product containing a certifiable antibiotic drug on which a request has been submitted in accordance with § 431.17, the fee for such service shall be the cost thereof. In such case the request shall be followed by an advance deposit in such amount as the Commissioner specifies, and thereafter such additional advance deposits shall be made as the Commissioner estimates may be necessary to prevent arrears in the payment of such fee.

(d) A person requiring continuing certification services may maintain an advance deposit of the estimated cost of such services for a two-month period. Such deposit shall be debited with fees for services rendered, but shall not be debited for any fee the amount of which is not definitely specified in the regulations in this chapter unless the depositor has previously requested the performance of the services to be covered by such fee. A monthly statement for each such advance deposit shall be rendered.

(e) The fees for the services rendered with respect to each batch certified under the regulations in this chapter shall accompany the request for certification, or the request for check tests and assays, unless such fee is covered by an advance deposit maintained in accordance with paragraph (d) of this section. Also, if the Commissioner considers that investigations other than examination of such samples are necessary to determine whether or not such batch complies with the requirements of § 431.10 for the issuance of a certificate, the fee shall include the cost of such investigations.

(f) The unearned portion of any advance deposit shall be refunded to the depositor upon his application.

(g) Whenever in the judgment of the Commissioner the ratio between fees collected (which are based upon experience and the best estimate of costs and the best estimate of earnings) and the costs of providing the service during an elapsed period of time, in the light of all circumstances and contingencies, warrants a refund from the fund collected during such period, he shall make ratable refunds to those persons to whom the services were rendered and charged, except for those services described under §§ 433.12 and 144.26 of this chapter.

(h) All deposits and fees required by the regulations in this chapter shall be paid by money order, bank draft, or certified check drawn to the order of the Food and Drug Administration, collectible at par at Washington, DC. All such deposits and fees shall be forwarded to the Food and Drug Administration, Department of Health, Education, and Welfare, Washington, DC, 20204, whereupon after making appropriate records thereof they will be transmitted to the Chief Disbursing Officer, Division of Disbursement, Treasurer of the United States, for deposit to the special account "Salaries and Expenses, Certification, Inspection, and Other Services, Food and Drug Administration."

Subpart C—Records and Reports

§ 431.60 Records and reports concerning experience with antibiotic drugs for human use for which a certificate or release has been issued.

(a) Each person engaged in manufacturing, compounding, processing, packing, or labeling any antibiotic drug for which a certificate or release has been issued or whose name appears on the labeling of an antibiotic drug as its manufacturer, packer, or distributor under a certificate or release shall establish and maintain such records and make such reports as are specified in this section to facilitate a determination whether any such certification or release should be rescinded or whether any regulation should be amended or repealed, including adequately organized and indexed files containing full reports of any information of the following kinds which is received or otherwise obtained by him from any source: *Provided, however,* That in order to avoid unnecessary duplication in the submission of reports, any such person's obligation to submit a report may be met by its submission on his behalf, designated as such, by another person responsible for reporting:

(1) Clinical experience, studies, investigations, and tests conducted by such person, or reported to him by any person, or reports in the scientific literature that are received or otherwise obtained by him, involving the drug that is the subject of the certificate or release or any related drug that is pertinent to the safety or efficacy thereof and which have not been previously submitted. An adequate summary and bibliography of literature reports will ordinarily suffice.

(2) Animal experience, studies, investigations, and tests conducted by such person, or reported to him by any person, or reports in the scientific literature that are received or otherwise obtained by him, involving the drug that is the subject of the certificate or release or any related drug that is pertinent to the safety or efficacy thereof and which have not been previously submitted. An adequate summary and bibliography of literature reports will ordinarily suffice.

(3) Experience, investigation, studies, or tests involving the microbiological, chemical, physical, biological properties, or any other properties of the drug, to the extent that such information is pertinent to its safety or efficacy, or to an evaluation of the adequacy of the methods, facilities, and controls used in its production and which have not been previously submitted.

(4) The information required by this section shall include, when known, adequate identification of its source, including the name and post-office address of the person who furnished such information.

(5) Copies of all mailing pieces and other labeling and, if it is a prescription drug, of all advertising used in promoting the drug and not previously submitted. A copy of the currently used package insert that gives full information for use of the drug shall accompany each submission of a report, whether or not such labeling has been previously submitted.

(6) Information concerning the quantity of the drug distributed, in a manner and form that facilitates estimates of the incidence of any adverse effects reported to be associated with the use of the drug. This does not require disclosure of financial or pricing data.

(b) Each person referred to in paragraph (a) of this section shall submit, in duplicate, to the Food and Drug Administration copies of the records and reports described in paragraph (a) of this section (except routine assay and control reports) appropriately identified with the antibiotic application number, if any, the name under which the drug is certified, and the number of the section in this chapter that provides for its certification, as follows. Each report for human-use drugs that forwards an advertisement or promotional labeling pursuant to paragraph (b)(3) of this section or a periodic report pursuant to paragraph (b)(4) of this section shall be accompanied by a completed transmittal Form FD–2253 (Transmittal of Advertisements and Promotional Labeling for Drugs for Human Use) or Form FD–2252 (Transmittal of Periodic Reports for Drugs for Human Use), respectively. Forms are obtainable from the Bureau of Drugs, Food and Drug Administration, Department of Health, Education, and Welfare, 5600 Fishers Lane, Rockville, MD 20852.

(1) Immediately upon receipt by such person, complete records or reports covering information of the following kinds:

(i) Information concerning any mix-up in the drug or its labeling with another article.

(ii) Information concerning any bacteriological, or any significant chemical, physical, or other change or deterioration in the drug, or any failure of one or more distributed batches of the drug to meet the specifications established for it by

regulations promulgated under section 507 of the act.

(2) As soon as possible, and in any event within 15 working days of its receipt by such person, complete records or reports concerning any information of the following kinds:

(i) Information concerning any unexpected side effect, injury, toxicity, or sensitivity reaction or any unexpected incidence or severity thereof associated with clinical uses, studies, investigations, or tests, whether or not determined to be attributable to the drug, except that this requirement shall not apply to the submission of information described in a written communication to such person from the Food and Drug Administration as types of information that may be submitted at other designated intervals. "Unexpected" as used in this paragraph (b)(2)(i) refers to conditions or developments not previously submitted as part of the request for certification or not encountered during clinical trials of the drug, or conditions or developments occurring at a rate higher than shown by information previously submitted as part of the request for certification or than encountered during such clinical trials.

(ii) Information concerning any unusual failure of the drug to exhibit its expected pharmacological activity.

(3) When mailing pieces, any other labeling, and advertising are devised for promotion of the drug, specimens shall be submitted at the time of initial dissemination of such labeling and at the time of initial publication of any such advertisement for a prescription drug. Mailing pieces and labeling that are designed to contain samples of a drug shall be complete except for omission of the drug.

(4) All the kinds of information described in paragraph (a) of this section, except that submitted under the provisions of paragraph (b)(1), (2), and (3) of this section, shall be submitted at the following intervals, unless otherwise ordered in a written communication from the Commissioner:

(i) If the drug is intended for administration to man, within intervals of 3 months beginning with the date of initial certification or release of a batch of the drug, during the first year following such date; within intervals of 6 months during the second year following such date; and at yearly intervals thereafter.

(ii) If the drug is intended solely for administration to animals, at intervals within 6 months beginning with the date of initial certification or release of a batch of the drug during the first year following such date, and at yearly intervals thereafter.

(iii) The submitted copies of records and reports shall include all the required information that was received or otherwise obtained by such person during the designated intervals.

(5) On written order of the Commissioner, within the time stated in such order or agreed to by such person and the Commissioner, any designated records or reports containing the kinds of information described in this section.

(c) The reports submitted under the provisions of this section are not required to furnish the names and addresses of individual patients unless the person referred to in paragraph (a) of this section is notified in writing by the Food and Drug Administration that individual patient identification is required with respect to designated reports in order to permit further investigation or because there is reason to believe that such reports do not represent actual results obtained.

(d) Any person referred to in paragraph (a) of this section shall upon request of any properly authorized officer or employee of the Department, at reasonable times, permit such officers to have access to and copy and verify any records and reports established and maintained under the provisions of this section.

(e) If the Food and Drug Administration finds that a person obtaining a certificate or release has failed to establish a system for maintaining required records, or has repeatedly or deliberately failed to maintain such records or to make required reports, in accordance with the provisions of this section, or that such person has refused to permit access to or copying or verification of such records or reports, the Commissioner shall give such person due notice and opportunity for a hearing on the question of whether to rescind a certificate or release.

(f) Upon written request of any person referred to in paragraph (a) of this section stating reasonable grounds therefor, the Commissioner will make available any information in possession of the Food and Drug Administration of the kinds such person is required to maintain under the provisions of this section, except information readily available to such person from other sources or information which the Commissioner concludes must be considered confidential.

§ 431.61 Records of distribution.

(a) The person who requested certification shall keep complete records showing each shipment and other delivery (including exports) of each certified batch or part thereof by such person or by any person subject to his control. Such records shall show the date and quantity of each such shipment or delivery and the name and post-office address of the person to whom such shipment or delivery was made, and shall be kept for not less than 3 years after such date.

(b) Upon the request of any officer or employee of the Food and Drug Administration, or of any other officer or employee of the United States acting on behalf of the Secretary, the person to whom a certificate is issued shall at all reasonable hours make such records available to any such officer or employee and shall accord to him full opportunity to make inventory of stocks of such batch on hand and otherwise to check the correctness of such records.

§ 431.62 Records retention.

At the option of the person having control of records required to be kept by any regulation in this Part 431, photostatic or other permanent reproductions may be substituted for such records after the first 2 years of the holding period.

PART 432—PACKAGING AND LABELING OF ANTIBIOTIC DRUGS

Sec.

432.1 Packaging requirements.
432.5 Labeling requirements.
432.9 Labeling of antibiotic drugs intended for export.
432.20 Declaration of potency.

AUTHORITY: Sec. 507, 59 Stat. 463, as amended (21 U.S.C. 357).

CROSS REFERENCE: For other regulations in this chapter concerning antibiotic drugs exempted from certain labeling requirements, see also § 1.107 of this chapter.

§ 432.1 Packaging requirements.

Each antibiotic drug subject to certification under section 507 or 512(n) of the act shall be packaged in immediate containers which shall be of such composition as not to cause any change in the strength, quality, or purity of the contents beyond any limits therefor in applicable standards, except that minor changes so caused that are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded. The immediate containers shall be tight containers as defined by the U.S.P., except that if the antibiotic drug is dispensed as an ointment or cream, the immediate containers shall be well-closed containers as defined by the U.S.P. If the antibiotic drug is packaged for dispensing, it may be packaged in combination with a container of a suitable and harmless diluent approved by the Commissioner.

(a) If it is a sterile preparation, the containers shall be sterile at the time of filling and closing and shall be so sealed that the contents cannot be used without destroying the seal.

(b) If it is intended for parenteral use, it shall be in containers of colorless, transparent glass (unless it is packaged to contain a single dose), closed by a substance through which a hypodermic needle may be introduced and withdrawn without removing the closure or destroying its effectiveness. Each container shall be filled with a volume in excess of that designated, which excess shall be sufficient to permit the withdrawal and administration of the volume, whether administered in single or multiple doses.

(c) If it is dispensed as a tablet, capsule, troche, pellet, or suppository, it may be enclosed in a foil or plastic film and such enclosure is a tight container as defined by the U.S.P., except for the provision that it shall be capable of tight reclosure. The immediate container may contain a dessicant separated from the

drug by a plug of cotton or other like material.

(d) If it is dispensed as an ointment or cream, it shall be in collapsible tubes which shall not be larger than the ½-ounce size if the ointment is represented for ophthalmic use, and in no case larger than the 2-ounce size, except that if it is labeled for institutional use, it may be packaged in immediate containers of glass.

(e) If it is intended for ophthalmic use or for inhalation therapy, the closure shall be one through which a hypodermic needle cannot be introduced.

§ 432.5 Labeling requirements.

(a) If an antibiotic drug is packaged for dispensing:

(1) It shall be labeled in accordance with the requirements prescribed by § 1.106(b) of this chapter, issued under section 502(f) of the act, unless the regulations pertaining to such drug specifically exempt it from such requirements.

(2) Its labeling shall bear any additional information required for the drug by specific regulations.

(3) Each package shall bear on its outside wrapper or container and the immediate container an expiration date prescribed for the drug by specific regulations; except that in lieu of the expiration date prescribed by specific regulations, a date may be used that is 12, 18, 24, 30, 36, 42, 48, 54, or 60 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that such drug as prepared by him is stable for such period of time. If the specific regulation does not stipulate an expiration period, it shall be as prescribed by this section. If the manufacturer or repacker of the drug has been exempted from the certification requirements, such date shall be the number of months after the month during which the batch was last assayed and released by the manufacturer or repacker. If an expiration date is used that is longer than the minimum date provided for the drug by specific regulations, it may be used only if the manufacturer has submitted information to the Commissioner adequate to prove that the drug is stable for such time.

(b) If it is packaged solely for manufacturing use or for repacking, each package shall bear on its outside wrapper or container and the immediate container, the following:

(1) The number of units or micrograms of activity per milligram or per gram, and the number of grams or kilograms in the immediate container.

(2) The batch mark.

(3) The statement "Caution: Federal law prohibits dispensing without prescription."

(4) The statement "For manufacturing use," "For repacking," or "For manufacturing use or repacking," and, if it is not sterile, the statement "nonsterile."

(5) The required expiration date.

(c) The expiration date prescribed for a drug by the regulations in this chapter may be omitted from the label of the immediate container if such container contains a single dose and it is packaged in an individual wrapper or container that bears the date prescribed.

§ 432.9 Labeling of antibiotic drugs intended for export.

(a) Antibiotic drugs subject to certification under section 507 of the act and intended for export will be certified notwithstanding failure to meet the labeling requirements of the applicable sections if the labeling used for such drugs meets the following conditions:

(1) It has been approved before use by the government authorities of the country to which the drugs are intended for export; and

(2) Such labeling represents that such drugs are for use only in those conditions for which they are certified for domestic distribution.

(b) The legend "Caution: Federal law prohibits dispensing without prescription" might be inappropriate on antibiotic drugs exported from the United States, since their sale may or may not be so restricted under the laws of the country of destination. The Food and Drug Administration would not object to a slight modification of the wording to read, "Caution: Federal (U.S.A.) law prohibits dispensing without prescription," by a manufacturer who wishes to market a drug under the same label both in domestic and foreign commerce.

§ 432.20 Declaration of potency.

Wherever the potency of an antibiotic drug included in the regulations in this chapter is expressed in terms of weight, such potency shall be equivalent to that contained in the same weight of the master standard of the drug.

PART 433—EXEMPTIONS FROM ANTIBIOTIC CERTIFICATION AND LABELING REQUIREMENTS

Subpart A—General Provisions

Sec.

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433.2 Conditions on the effectiveness of exemptions from certification.
433.3 Assay requirements for antibiotic drugs exempted from certification.

Subpart B—Exemptions for Which an Application or Notice Is Required

433.12 Exemption for labeling.
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433.20 Antibiotic drugs for isolation and differentiation of microorganisms in clinical use.
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Subpart D—Records and Reports

Sec.

433.30 Records retention.

AUTHORITY: Sec. 507, 59 Stat. 463, as amended (21 U.S.C. 357).

Subpart A—General Provisions

§ 433.1 Procedures for obtaining exemption from antibiotic drug certification requirements.

(a) The Commissioner finds that if the conditions of this section are met, the certification of antibiotic drugs for local or topical use will not be necessary to insure safety and efficacy of such drugs. The conditions are as follows:

(1) The antibiotic used in the manufacture of the drug has been marketed commercially in the United States as a drug for at least 5 years.

(2) The dosage form has been marketed commercially in the United States for at least 2 years.

(3) The drug is packaged and labeled for dispensing (drugs packaged for manufacturing use or for repacking are not eligible for exemptions under this section).

(4) The person seeking exemption has submitted to the Commissioner of Food and Drugs a petition establishing the following:

(i) The petitioner has used the certification services for the drug for not less than 12 months.

(ii) The petitioner has produced or repackaged and submitted for release or for certification the following number of batches of drugs within a period of time not exceeding 18 months, and no batch has failed to comply with its professed or prescribed standards of identity, strength, quality, or purity:

(*a*) Not less than 50 consecutive batches of the drug for which the petition is submitted; or

(*b*) Not less than 25 consecutive batches of such drug and not less than 25 consecutive batches of other associated antibiotic drugs of the same dosage form as the petitioned drug: *Provided, however,* That such other drugs contained among their ingredients the same antibiotic(s) as that (those) contained in the petitioned drug. (For example, if a manufacturer within 18 months has produced 25 acceptable batches of penicillin ointment and a total of 25 acceptable batches of penicillin-streptomycin ointment and penicillin-streptomycin-bacitracin ointment, his penicillin ointment could be considered for an exemption from certification.)

(iii) The petitioner, if he is the manufacturer of the drug, will use in the compounding of the exempt drug only batches of antibiotics that meet (as determined from samples tested by the Food and Drug Administration) the standards of identity, strength, quality, and purity specified for their use in the manufacture of such drug by the applicable regulations in this chapter.

(iv) The petitioner will perform, or will have performed for him, all the laboratory tests and assays required as a condition for certification on each batch produced and will not distribute batches

until such tests and assays have demonstrated that the exempt drug complies with the specifications for certification.

(v) The petitioner will maintain records of all the laboratory tests and assays referred to in paragraph (a)(4)(iv) of this section and make such records available to any officer or employee of the Food and Drug Administration at any reasonable time within 3 years after the date of the last shipment of each batch.

(vi) The petitioner will maintain records of distribution of batches and make them available for inspection on the same basis as is required for certified antibiotic dosage forms.

(b) In deciding whether such drug is to be exempted from such requirements, the Commissioner will give consideration, among other relevant factors, to:

(1) The possible effect of significant shortages or overages of the antibiotic in the drug.

(2) The stability of the drug.

(3) The results of a review made by an officer or employee of the Food and Drug Administration of all records required by regulations to be maintained for the drug by the petitioner.

(4) Results of examination of a market sample of the drug collected by the Food and Drug Administration from commercial channels.

(c) Exemption from batch certification will, in accordance with the provisions of these regulations, be applicable only to the petitioner requesting it and will not exempt any other person from the requirements of section 507 or 512n of the act.

(d) If the Commissioner concludes that the data in a petition, or otherwise before him, enable him to determine that the conditions for an exemption have been met, he will inform the petitioner that the requested exemption is granted. The Commissioner, upon granting any such exemption, will have published in the FEDERAL REGISTER a notice to that effect.

(e) If the Commissioner concludes that the conditions for an exemption specified in this section have not been met, he will inform the petitioner of his decisions and his reasons therefor.

(f) In accordance with the requirements of section 507(e) of the act, any antibiotic-containing drug for human use exempted under this section is deemed subject to section 505; and accordingly, the act of exempting the drug from the requirements of section 507 and 502(l) shall be considered also as constituting an act of approval of a new-drug application under section 505, thus subjecting the exempted antibiotic drug to the requirements of that section.

§ 433.2 Conditions on the effectiveness of exemptions from certification.

(a) If at any time after an exemption for an antibiotic drug has been granted, the Commissioner finds on the basis of new information before him with respect to such exempted drug, evaluated together with the evidence available to him when such exemption was granted, that certification of each batch is necessary to insure its safety and efficacy of use, he shall act immediately to revoke all exemptions granted for such drug.

(b) If the Commissioner finds that the person granted an exemption for an antibiotic drug for use in humans has failed to comply with the requirements of section 505 of the act and the regulations promulgated thereunder; or if he finds that the requirements of § 433.1 have not been met; or if he finds that the petition for exemption from batch certification contains any false statements of fact, the Commissioner may revoke such exemption immediately and require certification of the drug until such person shows adequate cause why such exemption should be reinstated.

(c) A hearing will be granted to any person who requests an opportunity to show that the exemption should be reinstated.

(d) If an exemption for an antibiotic drug or the approved new-drug application or an exemption granted to a petitioner is repealed or suspended by the Commissioner, a notice to that effect and the reasons therefor will be published in the FEDERAL REGISTER.

§ 433.3 Assay requirements for antibiotic drugs exempted from certification.

(a) Certain antibiotic drugs are exempted by regulations in this chapter from the certification requirements of sections 507 and 512 of the act if such drugs comply with standards prescribed by such regulations and on condition that the label of each package bears an expiration date which is determined from the date during which the batch was last assayed and released by the manufacturer.

(b) It is the position of the Food and Drug Administration that if each batch of such exempted drugs is not tested by the manufacturer or his agent to determine whether it complies with the standards of identity, strength, quality, and purity prescribed for it, the batch is not exempt from certification and it may be deemed to be misbranded under section 502(l) of the act or be adulterated under section 501(a)(5) of the act when in interstate commerce.

Subpart B—Exemptions for Which an Application or Notice is Required

§ 433.12 Exemption for labeling.

(a) Except as provided by paragraphs (c) and (d) of this section, a shipment or other delivery of a certifiable antibiotic drug which is to be labeled at an establishment located elsewhere than at the place of manufacture shall be exempt, during the time of introduction into and movement in interstate commerce and the time of holding in such establishment, from the requirement of section 502(l) of the act or the certification requirements of section 512(n) of the act if the labeling of each shipping container bears the batch mark of the drug, the number of units per package and the expiration date, and if the person who introduced such shipment or delivery into interstate commerce holds a permit (Antibiotic Form 3) from the Commissioner authorizing shipment for labeling in such establishment.

(b) (1) An application for such a permit shall be in a form specified by the Commissioner and shall give the name and location of the establishment in which such labeling is to be done.

(2) In case the applicant is the operator of such establishment, the application shall include a written agreement signed by him that he will request certification of each batch from which any shipment or delivery is made to such establishment unless it is exempt under section 801(d) of the act or § 433.17; that he will not remove any of such antibiotic drug from such establishment unless it complies with section 502(l) of the act or the certification requirements of section 512(n) of the act or is so exempt, or if certification is refused, unless it is returned within a reasonable time to permit reprocessing and certification, destruction, or such exemption at the establishment where it was manufactured; that he will keep complete records showing the date, quantity, and batch mark of each such shipment and delivery and the disposition thereof; that he will make such records available to any officer or employee of the Food and Drug Administration at any reasonable hour within 3 years after the date of such disposition; and that he will accord full opportunity to such officer or employee to make inventories of stocks on hand and otherwise check the correctness of such records.

(3) In case the applicant is not the operator of such establishment such application shall include or be accompanied by:

(i) A written agreement signed by the applicant that he will request certification of each batch from which any shipment or delivery is made to such establishment unless it is exempt under section 801(d) of the act or § 433.17; that he will keep complete records showing the date, quantity, and batch mark of each such shipment and delivery; and that he will make such records available to any officer or employee of the Food and Drug Administration at any reasonable hour within 3 years after the date of such shipment or delivery; and

(ii) A written agreement signed by the operator of such establishment that he will submit a request, supplemental to that of the applicant, for the certification of each batch or portion thereof comprised in any such shipment or delivery received by him unless it is exempt under section 801(d) of the act or § 433.17; that he will specify in his request the number of packages of each size in such shipment or delivery, the date of delivery, the batch mark thereof, and the batch mark he will use therefor; that the batch marks to be used (if different from those of the applicant) will be only those of which the key is specified in this agreement that the expiration date used for the batch will be only that assigned to the manufacturer by certification; that the labeling to be used for such packages will be only that of which specimens are attached to this

agreement (including specimens of all brochures and other printed matter, except readily available medical publications, referred to in such labeling); that when any change is made in such key or labeling he will promptly submit to the Commissioner a full statement of such change or, in the case of changed labeling, specimens showing all such changes; that he will not remove any of such antibiotic drug from such establishment unless it complies with section 502(l) of the act or is exempt under section 801(d) of the act or § 433.17 or, if certification is refused, unless it is returned within a reasonable time to permit reprocessing and certification, destruction, or such exemption at the establishment where it was manufactured; that he will keep complete records of the disposition of each such shipment and delivery; that he will make such records available to any officer or employee of the Food and Drug Administration at any reasonable hour within 3 years after the date of such disposition; and that he will accord full opportunity to such officer or employee to make inventories of stocks on hand and otherwise check the correctness of such records.

(4) When the Commissioner finds, after giving notice and opportunity for hearing, that such application contains any untrue statement of a material fact or that any provision of any such agreement has been violated he may revoke such permit.

(c) An exemption of a shipment or other delivery under paragraph (a) of this section, in case the person who introduced such shipment or delivery into interstate commerce is the operator of such establishment, shall become void at the beginning of the act of removing or offering to remove such shipment or delivery or any part thereof, before or after labeling, from such establishment unless such batch complies with section 502(l) of the act or the certification requirements of section 512(n) of the act or is exempt under section 801(d) of the act or § 433.17 or, if certification is refused, unless such shipment or delivery is returned within a reasonable time to permit reprocessing and certification, destruction, or such exemption at the establishment where it was manufactured.

(d) An exemption of a shipment or other delivery under paragraph (a) of this section, in case the person who introduced such shipment or delivery into interstate commerce is not the operator of such establishment, shall expire at the beginning of the act of removing or offering to remove such shipment or delivery of any part thereof, before or after labeling from such establishment unless such batch complies with section 502(l) of the act or the certification requirements of section 512(n) of the act or is exempt under section 801(d) of the act or § 433.17 or, if certification is refused, unless such shipment or delivery, within a reasonable time, is destroyed or returned to permit reprocessing and certification, destruction, or such exemption at the establishment where it was manufactured.

§ 433.13 Exemption for manufacturing use.

(a) Except as provided by paragraphs (c) and (d) of this section, a shipment or other delivery of any certifiable antibiotic drug subject to the regulations in this chapter that is packed in containers of not less than 10,000,000 units of penicillin or 10 grams each of one of the other antibiotic drugs shall be exempt, during the time of introduction into and movement in interstate commerce and the time of holding in the establishment where it is so used, from the requirements of section 502(l) of the act or the certification requirements of section 512(n) of the act, if it conforms to the standards prescribed therefor by the section of the regulations in this chapter which is specifically applicable to such other antibiotic drug, if the label of each container bears the batch mark of the drug, the number of units or grams per package, and the date on which the latest assay of the drug was completed, and if the person who introduced each shipment or delivery into interstate commerce holds a permit from the Commissioner authorizing shipment for manufacturing use in such establishment.

(b) An application for such a permit shall be in a form specified by the Commissioner, shall give the name and location of the establishment in which such drug is to be used and shall be accompanied by;

(1) A written agreement signed by the applicant that he will keep complete records showing the date, quantity, and batch mark of each shipment and other delivery of any such drug to such establishment, and that he will make such records available to any officer or employee of the Food and Drug Administration at any reasonable hour within 3 years after the date of such shipment or delivery;

(2) A written statement signed by the operator of such establishment showing that he has adequate facilities for the manufacture of such other drug; such statement shall contain an agreement that he will keep complete records showing the date of receipt by him and the quantity and batch mark of each such shipment and delivery and the disposition thereof and showing the quantity and batch mark of each batch of such other drug manufactured by him and the disposition thereof; that he will make such records available to any officer or employee of the Food and Drug Administration at any reasonable hour within 3 years after the date of such disposition, and that he will accord full opportunity to such officer or employee to make inventories of stocks on hand and otherwise check the correctness of such records; and

(3) A written agreement signed by the person who will own the drug after its manufacture is completed that he will request certification of each batch thereof unless it is exempt under section 801 (d) of the act or §§ 433.12, 433.14, 433.16, or 433.17, and that he will not remove any of such drug from such establishment unless it complies with section 502(l) of the act or the certification requirements of section 512(n) of the act or is so exempt or is returned to him for labeling.

When the Commissioner finds, after giving notice and opportunity for hearing, that such application contains any untrue statement of a material fact or that any provision of any such agreement has been violated he may revoke such permit.

(c) An exemption of a shipment or other delivery under paragraph (a) of this section, in case the person who introduced such shipment or delivery into interstate commerce is the operator of such establishment, shall become void at the beginning of the act of removing or offering to remove such shipment or delivery or any part thereof from such establishment, prior to its use in the manufacture of another drug, unless it is exempt under section 801(d) of the act.

(d) An exemption of a shipment or other delivery under paragraph (a) of this section, in case the person who introduced such shipment or delivery into interstate commerce is not the operator of such establishment, shall expire at the beginning of the act of removing or offering to remove such shipment or delivery or any part thereof from such establishment, prior to its use in the manufacture of another drug, unless it is exempt under section 801(d) of the act.

§ 433.14 Exemption for storage.

(a) Except as provided by paragraphs (c) and (d) of this section, a shipment or other delivery of a drug which is to be stored at a warehouse located elsewhere than at the place of manufacture shall be exempt, during the time of introduction into and movement in interstate commerce and the time of holding in such warehouse, from the requirements of section 502(l) of the act or the certification requirements of section 512(n) of the act if the labeling of each shipping container bears the batch mark of the drug, and if the person who introduced such shipment or delivery into interstate commerce holds a permit from the Commissioner authorizing shipment for storage in such warehouse.

(b) An application for such a permit shall be in a form specified by the Commissioner, and shall give the name and location of the warehouse in which such drug is to be stored. Such application shall be accompanied by:

(1) A written agreement signed by the applicant that he will request certification of each batch thereof unless it is exempt under section 801(d) of the act or §§ 433.12, 433.13, or 433.16, that he will not remove any of such drug from such warehouse unless it complies with section 502(l) of the act or the certification requirements of section 512(n) of the act or is so exempt or, if certification is refused unless it is returned within a

reasonable time to permit reprocessing and certification, destruction, or such exemption at the establishment where it was manufactured; that he will keep complete records showing the date, quantity, and batch mark of each shipment and other delivery of any such drug to such warehouse, and that he will make such records available to any officer or employee of the Food and Drug Administration at any reasonable hour within 3 years after the date of such shipment or delivery; and

(2) A written statement signed by the operator of such warehouse showing that he has adequate facilities for such storage; such statement shall contain an agreement that he will hold each shipment or other delivery of such drug intact, under such conditions as will not cause failure of the drug to comply with the requirements for certification, that he will keep complete records showing the date of receipt by him and the quantity and batch mark of each such shipment and delivery and the disposition thereof, that he will make such records available to any officer or employee of the Food and Drug Administration at any reasonable hour within 3 years after the date of such disposition, and that he will accord full opportunity to such officer or employee to make inventories of stocks on hand and otherwise check the correctness of such records.

If the applicant keeps complete records showing the date, quantity, and batch mark of each shipment and other delivery of any such drug from such warehouse and the name and post-office address of the person to whom such shipment or delivery was made, the agreement to keep records of such disposals, to make such records available, and to afford opportunity for checking their correctness may be included in the applicant's agreement and omitted from that of the operator. When the Commissioner finds, after giving notice and opportunity for hearing, that such application contains any untrue statement of a material fact or that any provision of any such agreement has been violated he may revoke such permit.

(c) An exemption of a shipment or other delivery under paragraph (a) of this section, in case the person who introduced such shipment or delivery into interstate commerce is the operator of such warehouse, shall become void at the beginning of the act of removing or offering to remove such shipment or delivery or any part thereof from such warehouse unless such batch complies with section 502(l) of the act or the certification requirements of section 512(n) of the act or is exempt under section 801(d) of the act or §§ 433.12, 433.13, or 433.16, or, if certification is refused, unless such shipment or delivery is returned within a reasonable time to permit reprocessing and certification, destruction, or such exemption at the establishment where it was manufactured.

(d) An exemption of a shipment or other delivery under paragraph (a) of this section, in case the person who introduced such shipment or delivery into interstate commerce is not the operator of such warehouse, shall expire at the beginning of the act of removing or offering to remove such shipment or delivery or any part thereof from such warehouse unless such batch complies with section 502(l) of the act or the certification requirements of section 512(n) of the act or is exempt under section 801(d) of the act or §§ 433.12, 433.13, or 433.16, or, if certification is refused, unless such shipment or delivery within a reasonable time, is destroyed, or returned to permit reprocessing and certification, destruction, or such exemption at the establishment where it was manufactured.

§ 433.15 Exemption for processing.

(a) Except as provided by paragraphs (c) and (d) of this section, a shipment or other delivery of any certifiable antibiotic drug subject to the regulations in this chapter in concentrated aqueous solution which is to be processed at an establishment located elsewhere than at the place of manufacture shall be exempt during the time of introduction into and movement in interstate commerce and the time of holding in such establishment from the requirements of section 502(l) of the act or the certification requirements of section 512(n) of the act, if the person who introduced such shipment or delivery into interstate commerce holds a permit from the Commissioner authorizing shipment for processing in such establishment, and each package of such solution bears the batch mark of the drug.

(b) An application for such a permit shall be in a form specified by the Commissioner and shall give the name and location of the establishment in which such processing is to be done. Such application shall be accompanied by:

(1) A written agreement signed by the applicant that he will keep complete records showing the date, quantity, potency, and batch mark of each shipment and other delivery of any such solution to such establishment, and that he will make such records available to any officer or employee of the Food and Drug Administration at any reasonable hour within 3 years after the date of such shipment or delivery;

(2) A written agreement signed by the operator of such establishment showing that he has adequate facilities for such processing; such statement shall contain an agreement that he will keep complete records showing the date of receipt by him and the quantity and batch mark of each such shipment and delivery and the disposition thereof, that he will make such records available to any officer or employee of the Food and Drug Administration at any reasonable hour within 3 year after the date of such disposition, and that he will accord full opportunity to such officer or employee to make inventories of stocks on hand and otherwise check the correctness of such records; and

(3) A written agreement signed by the person who will own the drug after the processing is completed that he will request certification of each batch thereof unless it is exempt under section 801(d) of the act or §§ 433.12, 433.13, 433.14, 433.16, or 433.17, and that he will not remove any of such drug from such establishment unless it complies with section 502(l) of the act or the certification requirements of section 512(n) of the act or is so exempt.

When the Commissioner finds, after giving notice and opportunity for hearing, that such application contains any untrue statement of a material fact or that any provision of any such agreement has been violated he may revoke such permit.

(c) An exemption of a shipment or other delivery under paragraph (a) of this section, in case the person who introduced such shipment or delivery into interstate commerce is the operator of such establishment, shall become void at the beginning of the act of removing or offering to remove such shipment or delivery or any part thereof, before or after processing, from such establishment unless the batch made from such shipment or delivery complies with section 502(l) or the certification requirements of section 512(n) of the act or is exempt under section 801(d) of the act or §§ 433.12, 433.13, 433.14, 433.16, or 433.17, or, if certification is refused, unless such shipment or delivery is reprocessed and certified or destroyed within a reasonable time.

(d) An exemption of a shipment or other delivery under paragraph (a) of this section, in case the person who introduced such shipment or delivery into interstate commerce is not the operator of such establishment, shall expire at the beginning of the act of removing or offering to remove such shipment or delivery or any part thereof, before or after processing, from such establishment unless the batch made from such shipment or delivery complies with section 502(l) of the act or is exempt under section 801(d) of the act or the certification requirements of section 512(n) of the act or §§ 433.12, 433.13, 433.14, 433.16, or 433.17, or, if certification has been refused, unless such shipment or delivery is reprocessed and certified or destroyed within a reasonable time.

§ 433.16 Exemption for repacking.

(a) Except as provided by paragraphs (c) and (d) of this section, a shipment or other delivery of a drug which is to be repacked at an establishment located elsewhere than at the place of manufacture shall be exempt, during the time of introduction into and movement in interstate commerce and the time of holding such establishment from the requirements of section 502(l) of the act or the certification requirements of section 512(n) of the act if the labeling of each container bears the batch mark of the drug and the number of units per package, and if the person who introduces such shipment or delivery into interstate commerce holds a permit from the Commissioner authorizing shipment for repacking in such establishment.

(b) An application for such a permit shall be in a form specified by the Com-

missioner, and shall give the name and location of the establishment in which such repacking is to be done. Such application shall be accompanied by:

(1) A written agreement signed by the applicant that he will keep complete records showing the date, quantity, and batch mark of each shipment and other delivery of any such drug to such establishment, and that he will make such records available to any officer or employee of the Food and Drug Administration at any reasonable hour within 3 years after the date of each shipment or delivery;

(2) A written statement signed by the operator of such establishment showing that he has adequate facilities for such repacking; such statement shall contain an agreement that he will keep complete records showing the date of receipt by him and the quantity and batch mark of each such shipment and delivery and the disposition thereof, that he will make such records available to any officer or employee of the Food and Drug Administration at any reasonable hour within 3 years after the date of such disposition, and that he will accord full opportunity to such officer or employee to make inventories of stocks on hand and otherwise check the correctness of such records; and

(3) A written agreement signed by the person who will own the drug after the repacking is completed that he will request certification of each batch thereof unless it is exempt under section 801(d) of the act or §§ 433.12, 433.13, 433.14, or 433.17, and that he will not remove any of such drug from such establishment unless it complies with section 502(l) of the act or the certification requirements of section 512(n) of the act or is so exempt or is returned to him for labeling or, if certification is refused, unless it is returned within a reasonable time to permit reprocessing and certification, destruction, or such exemption at the establishment where is was manufactured.

When the Commissioner finds, after giving notice and opportunity for hearing, that such application contains any untrue statement of a material fact or that any provision of any such agreement has been violated he may revoke such permit.

(c) An exemption of a shipment or other delivery under paragraph (a) of this section, in case the person who introduced such shipment or delivery into interstate commerce is the operator of such establishment, shall become void at the beginning of the act of removing or offering to remove such shipment or delivery or any part thereof, before or after repacking, from such establishment unless such batch complies with section 502 (l) of the act or the certification requirements of section 512(n) of the act or is exempt under section 801(d) of the act or §§ 433.12, 433.13, 433.14, or 433.17, or is returned to such person for labeling or, if certification is refused, unless such shipment or delivery is returned within a reasonable time to permit reprocessing and certification, destruction, or such exemption at the establishment where it was manufactured.

(d) An exemption of a shipment or other delivery under paragraph (a) of this section, in case the person who introduced such shipment or delivery into interstate commerce is not the operator of such establishment, shall expire at the beginning of the act of removing or offering to remove such shipment or delivery or any part thereof, before or after repacking, from such establishment unless such batch complies with section 502(l) of the act or the certification requirements of section 512(n) of the act or is exempt under section 801(d) of the act or §§ 433.12, 433.13, 433.14, or 433.17, or is returned to such person for labeling or, if certification is refused, unless such shipment or delivery within a reasonable time, is destroyed or returned to permit reprocessing and certification, destruction, or such exemption at the establishment where it was manufactured.

§ 433.17 Exemption for investigational use.

A shipment or other delivery of an antibiotic drug shall be exempt from section 502(l) of the act or the certification requirements of section 512(n) of the act if all the procedures outlined in § 312.1 or § 135.3 of this chapter are complied with. For the purposes of this section, the references in § 312.1 or § 135.3 of this chapter to "new drug" and "approved new animal drug application" shall be deemed to read "antibiotic drug" and "approval for certification or exemption from certification" respectively.

Subpart C—Specific Use Exemptions

§ 433.20 Antibiotic drugs for isolation and differentiation of microorganisms in clinical use.

Antibiotic drugs subject to section 507 of the act shall be exempt from section 502(l) if such drugs are:

(a) Paper discs impregnated with antibiotics in the amounts listed in the following table:

Antibiotic	*Content per Disc*
Bacitracin	0.04 unit.
Nystatin	100 units.

(b) Packaged in a container bearing on its label or labeling the following:

(1) On the outside wrapper or container and the immediate container:

(i) The batch mark.

(ii) The potency of each disc in the batch.

(iii) The expiration date as prescribed under § 432.5(a)(3) of this chapter.

(iv) The statement: Not for Susceptibility Testing.

(2) On the labeling within or attached to the package: Adequate directions for use.

§ 433.21 Antibiotics for diagnostic use.

Antibiotics packaged for the withdrawal of individually weighed portions and intended for use solely in laboratory procedures in connection with the diagnosis or treatment of disease and conspicuously so labeled shall be exempt from the certification requirements of section 502(l) and 507 of the act and the certification requirements of section 512(n) of the act if they comply with all the following conditions:

(a) The potency, moisture content, and identity comply with the standards prescribed for the antibiotic by the specific regulations issued in this chapter.

(b) It is packaged in immediate containers that are tight containers as defined by the U.S.P. Each such container shall contain not more than 1 gram.

(c) Each package bears on the label or labeling of its outside wrapper or container and the immediate container the following:

(1) The statements "For the withdrawal of individual portions of antibiotic. Each portion must be weighed before use. Diagnostic reagent. For professional use only."

(2) The number of milligrams or grams contained in each immediate container and the potency per milligram.

(3) The batch mark.

(4) The statement "Expiration date _____", the blank being filled in with the date that does not exceed the expiration date authorized for the antibiotic by this chapter.

(d) The circular or other labeling within or attached to the package bears directions adequate for the use of such drug.

CROSS REFERENCES: For tests and methods of assay and certification of antibiotic sensitivity discs for laboratory diagnosis of disease, see §§ 460.1 and 460.6 of this chapter.

§ 433.22 Biologic drugs that contain antibiotics as a preservative.

Biological drugs that contain any certifiable antibiotic drug subject to the regulations in this chapter, and the purpose of the antibiotic is for use only as a preservative and the biological drug is conspicuously so labeled, shall be exempt from the requirements of sections 502 (l) and 507 of the act and the certification requirements of section 512(n) of the act, if such drugs are licensed under the Public Health Service Act of July 1, 1944 (58 Stat. 682; 42 U.S.C. 201 et seq.) or under the Virus-Serum-Toxin Act of March 4, 1913 (37 Stat. 832; 21 U.S.C. 151 et seq.).

§ 433.23 Microbiological culture media containing antibiotics.

Microbiological culture media that contain any certificable antibiotic drug subject to the regulations in this chapter shall be exempt from the requirements of sections 502(l) and 507 of the act and the certification requirements of section 512(n) of the act if:

(a) They are intended for use in tissue culture and the antibiotic drug is added solely for use as an aid in the prevention of microbial contamination; or

(b) They are intended for use in the isolation of selected organisms from mixed cultures and the antibiotic drug is added solely for use as an aid in such isolation; and

(c) The certifiable antibiotic drug used in such culture media complies with

the applicable standards of identity, strength, quality, and purity prescribed therefor.

§ 433.24 Exemption of antibiotic drugs for use in teaching, law enforcement, research, and analysis.

Antibiotic drugs subject to section 507 or 512(n) of the act shall be exempt from the requirements of section 502(l) and from the certification requirements of section 512(n) of the act if shipped or sold to, or in the possession of, persons regularly and lawfully engaged in instruction in pharmacy, chemistry, or medicine not involving clinical use; or in law enforcement; or in research not involving clinical use; or in chemical analysis or physical testing, provided they are to be used only for such instruction, law enforcement, research, analysis, or testing, and provided further that their labels bear the statement "Not for drug use."

§ 433.25 Antibiotic drugs intended for export.

(a) Unless exempted pursuant to section 507(c) or 512(n)(3) of the Federal Food, Drug, and Cosmetic Act, antibiotic drugs consigned to persons engaged in export shipment of the articles are fully subject to the certification requirements of sections 502(l) and 507 of the act or of section 512(n) of the act. Further, unless exempted pursuant to section 507(d), 512(n)(4), or 801(d) of the act, such shipments are required to be labeled in full conformance with the act and regulations promulgated thereunder. In order to qualify for exemption under section 801(d) of the act, the drugs, at the time of introduction into interstate commerce, must be marked for export, must accord to the specifications of the foreign purchaser, and must not be in conflict with the laws of the country to which they are intended for export. The initial shipper has a responsibility to meet all the conditions of section 801(d) if exemption is to be claimed.

(b) Section 433.12 provides exemption from labeling. If the original shipper has an effective exemption permit under the regulations in this Part 433, the exporting firm named in the exemption permit may be allowed to receive and to hold the unlabeled drugs under the exemption until the conditions of section 801(d) are satisfied.

Subpart D—Records and Reports

§ 433.30 Records retention.

At the option of the person having control of records required to be kept by any regulation in this Part 433, photostatic or other permanent reproductions may be substituted for such records after the first 2 years of the holding period.

PART 436—TESTS AND METHODS OF ASSAY OF ANTIBIOTIC AND ANTIBIOTIC-CONTAINING DRUGS

Subpart A—Definitions; Interpretations; Requirements

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436.1 Sterility requirements of items packaged with sterile antibiotic drugs.
436.2 Alternative assay methods.

Subpart B—Sterility Test Methods

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436.20 Sterility test methods and procedures.

Subpart C—Biological Test Methods

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436.200 Loss on drying.
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Subpart F—Chemical Tests for Specific Antibiotics

436.300 Polarimetric assay of carbenicillin indanyl sodium.
436.301 Thin layer chromatography identity test for carbenicillin indanyl.
436.302 Clindamycin vapor phase chromatography.
436.303 Clindamycin content of clindamycin palmitate hydrochloride by vapor phase chromatography.
436.304 Clindamycin phosphate vapor phase chromatography.
436.305 Thin layer chromatographic identity test for hetacillin.
436.306 Lincomycin vapor phase chromatography.
436.307 Spectinomycin vapor phase chromatography.
436.308 Paper chromatography identity test for tetracyclines.
436.309 Anhydrotetracyclines and 4-epianhydrotetracycline.
436.310 Thin layer chromatography identity test for mitomycin.

Subpart G—Chemical Tests for Nonantibiotic Active Ingredients

436.400 Thin layer chromatographic identity test for iodochlorhydroxyquin.

Subpart H—Tests for Specific Antibiotic Dosage Forms

436.500 Penicillin in oil and wax.
436.503 Procaine penicillin and buffered crystalline penicillin for aqueous injection.
436.504 Penicillin-bacitracin ointment.
436.505 Penicillin-streptomycin-bacitracin ointment; penicillin-dihydrostreptomycin-bacitracin ointment; penicillin-streptomycin-bacitracin methylene disalicylate ointment; penicillin-dihydrostreptomycin-bacitracin methylene disalicylate ointment.
436.506 Benzathine penicillin G and buffered crystalline penicillin for aqueous injection.
436.507 Benzathine-procaine-buffered crystalline penicillins for aqueous injection.
436.508 Penicillin - bacitracin - neomycin ointment; penicillin-bacitracin-neomycin in oil.
436.509 Procaine penicillin-streptomycin-polymyxin in oil; procaine penicillin-dihydrostreptomycin-polymyxin in oil; procaine penicillin-streptomycin-polymyxin ointment; procaine penicillin-dihydro-streptomycin-polymyxin ointment.
436.510 Penicillin - streptomycin - erythromycin ointment; penicillin-dihydro-streptomycin - erythromycin ointment.
436.511 Penicillin-streptomycin-bacitracin methylene disalicylate-neomycin ointment; penicillin-dihydrostreptomycin-bacitracin methylene disalicylate-neomycin ointment.
436.512 Procaine penicillin G-novobiocin-neomycin-dihydrostreptomycin in oil.
436.513 Chlortetracycline troches; tetracycline hydrochloride troches.
436.514 Chlortetracycline hydrochloride powder topical; tetracycline hydrochloride powder topical.
436.515 Capsules tetracycline and oleandomycin phosphate; capsules tetracycline and troleandomycin; capsules tetracycline hydrochloride and oleandomycin phosphate; capsules tetracycline hydrochloride and troleandomycin.
436.516 Tetracycline-neomycin complex powder topical; tetracycline hydrochloride-neomycin sulfate powder topical.
436.517 Bacitracin-neomycin tablets; zinc bacitracin-neomycin tablets; bacitracin methylene disalicylate-neomycin tablets.

AUTHORITY: Sec. 507, 59 Stat. 463, as amended (21 U.S.C. 357), unless otherwise noted.

Subpart A—Definitions; Interpretations; Requirements

§ 436.1 Sterility requirements of items packaged with sterile antibiotic drugs.

(a) *Diluents packaged in combination with sterile antibiotic drugs.* If a sterile antibiotic drug is packaged in combination with an immediate container of a diluent, the immediate container of diluent shall be sterile when tested by the method prescribed in § 436.20(e)(1).

(b) *Droppers packaged in combination with sterile antibiotic drugs.* If a sterile antibiotic drug is packaged in combination with a dropper, such dropper shall be sterile when tested by the method prescribed in § 436.20(e)(1).

§ 436.2 Alternative assay methods.

Alternative assay methods (including automated procedures) employing the same basic chemistry or microbiology as the official methods described in this part and in the individual monographs of this chapter may be used, provided the results obtained are of equivalent accuracy. However, only the results obtained from the official methods designated in the individual monographs are conclusive.

Subpart B—Sterility Test Methods

§ 436.20 Sterility test methods and procedures.

(a) *Laboratory facilities.* The test must be performed using aseptic techniques in an area as free from contamination as is possible to achieve. Testing should not be conducted under direct exposure to ultraviolet light or in areas under aerosol treatment. Environmental tests to assess the suitability of testing conditions should be made frequently enough to assure the validity of test results.

(b) *Equipment and reagents*—(1) *Bacterial membrane filter.* The filter has a nominal porosity of 0.45 micron±0.02 micron, a diameter of approximately 47 millimeters, and a flowrate of 55 milliliters to 75 milliliters of distilled water passing each square centimeter of filter area per minute with a differential pressure of 70 centimeters of mercury at 25° C.

(2) *Penicillinase solutions.* When the amount of penicillinase to be used is specified in terms of Levy units, use a penicillinase solution standardized in terms of Levy units. One Levy unit of penicillinase inactivates 59.3 units of penicillin G in 1 hour at 25° C. and at a pH of 7.0 in a phosphate buffered solution of a pure alkali salt of penicillin G when the substrate is in sufficient concentration to maintain a zero order reaction.

(c) *Culture media.* Use ingredients that conform to the standards prescribed by the U.S.P. or *N.F.* In lieu of preparing the media from the individual ingredients, they may be made from dehydrated mixtures which, when reconstituted with distilled water, have the same or equivalent composition as such media and have growth-promoting buffering, and oxygen tension-controlling properties equal to or better than such media. The pH of each medium should be adjusted with 2*N* hydrochloric acid or sodium hydroxide before sterilization, so that after sterilization and the addition of the penicillinase, if necessary, the pH will fall within the specified range. Dispense 90±10 milliliter quantities of the liquid media into individual test tube (38 millimeters x 200 millimeters). Close the tubes with suitable closures, and sterilize in an autoclave at 121° C. for 20 minutes. The autoclave temperature should be reached within 10 minutes. After sterilization, cool the medium at once to approximately 25° C. and store at 20° C. to 30° C. The sterility of each lot of tubes of liquid medium may be confirmed by incubating an adequate number of tubes as described in the test procedures in paragraph (e) of this section.

(1) *Medium A.* Use U.S.P. fluid thioglycolate medium I.

(2) *Medium B.* Use U.S.P. fluid thioglycolate medium I, with sufficient sterile penicillinase added to inactivate the penicillin activity in the sample under test. The penicillinase must be added to individual tubes of sterile medium A, using aseptic technique. Prior to use, or at the time of the test, a representative number of the tubes containing added penicillinase are incubated at 30° C.–32° C. for 24 hours to 48 hours, and are examined for sterility. If the sample contains penicillin as the only antibiotic, the ability of the penicillinase to inactivate all the penicillin in the sample under test is checked as follows: Add to one test tube of medium B the proper amount of penicillin from one of the individual containers under test. Then add 1.0 milliliter of a 1:1,000 dilution of an 18–24 hour culture of *Staphylococcus aureus* (American Type Culture Collection 6538–P)[1] in medium A. Typical microbial growth must be observable after 24 hours incubation at 30° C.–32° C. If the sample contains a mixture of penicillin plus some other antibiotic or antibacterial agent, the ability of the penicillinase to inactivate all the penicillin in the sample is not tested directly on the sample under test, but is determined separately, using an amount of penicillin alone equivalent to the amount of penicillin in the sample or by any other suitable method for standardizing the penicillin-inactivating power of the penicillinase preparation.

(3) *Medium C.* To each liter of medium A add 5.0 milliliters of polysorbate 80 before sterilization. To each tube of sterilized medium add sufficient sterile penicillinase, and proceed as directed for medium B.

(4) *Medium D.* To each liter of medium A add 5.0 milliliters of polysorbate 80 and sufficient 2*N* sodium hydroxide so that the pH will be 7.9±0.1 after sterilization. Then add sufficient sterile penicillinase to each tube and proceed as directed for medium B.

(5) *Medium E.* Use U.S.P. XVIII soybean-casein digest medium.

(6) *Medium F.* To each liter of medium E add 5.0 milliliters of polysorbate 80 before sterilization. To each tube of sterilized medium add sufficient sterile penicillinase to solubilize the penicillin in the sample to be tested.

(7) *Medium G.* Prepare as follows:

Peptic digest of animal tissue	6.0 gm.
Pancreatic digest of casein	4.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Dextrose	1.0 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 6.6±0.1.

Suspend the powder in a liter of distilled water. Allow to stand for 5 minutes, then mix thoroughly. Boil for 1 or 2 minutes or until solution is complete. Dispense in suitable flasks and sterilize at 121° C. for 15 minutes. Aseptically pour approximately 25-milliliter quantities into sterile Petri dish bottoms measuring 20 millimeters x 100 millimeters. Cover plates with sterile porcelain tops, glazed on the outside. Allow plates to stand at room temperature for 48 hours prior to use as a control on the sterility of the plates.

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

(8) *Medium H.* Prepare, sterilize, and dispense as described for medium G, except as follows:

Dextrose	40.0 gm.
Peptic digest of animal tissue	10.0 gm.
Agar	15.0 gm.
Distilled, water, q.s.	1,000.0 ml.

pH 5.6±0.1 after sterilization.

(9) *Medium I.* To each liter of Medium A add 1 milliliter of *p-tert*-octylphenoxy polyethoxyethanol.

(10) *Medium J.* To each liter of Medium E add 1 milliliter of *p-tert*-octylphenoxy polyethoxyethanol.

(11) *Medium K.* (*Rinse medium*). Prepare as follows:

Peptic digest of animal tissue: 5.0 gm.
Beef extract: 3.0 gm.
p-tert-octylphenoxy polyethoxyethanol: 10.0 ml.
Distilled water, q.s.: 1,000.0 ml.
pH 6.9±0.2 after sterilization.

(12) *Medium L.* To each liter of Medium A add 1 milliliter of *p-tert*-octylphenoxy polyethoxyethanol and approximately 10,000 Levy units of penicillinase.

(13) *Medium M.* To each liter of Medium E add 1 milliliter of *p-tert*-octylphenoxy polyethoxyethanol and approximately 10,000 Levy units of penicillinase.

(d) *Diluting fluids*—(1) *Diluting fluid A.* Dissolve 1 gram of U.S.P. peptic digest of animal tissue or equivalent in sufficient distilled water to make 1,000 milliliters. Dispense in flasks and sterilize as described in paragraph (c) of this section. Final pH=7.1±0.1.

(2) *Diluting fluid B.* To each liter of diluting fluid A add 5.0 milliliters of polysorbate 80 before sterilization.

(3) *Diluting fluid C.* To each liter of diluting fluid A add 0.5 gram of sodium thioglycollate, and adjust with NaOH so that after sterilization the final pH will be pH 6.6±0.6. Dispense in flasks and sterilize as described in paragraph (c) of this section.

(4) *Diluting fluid D.* To each liter of diluting fluid A add 1 milliliter of *p-tert*-octylphenoxy polyethoxyethanol. Dispense in flasks and sterilize as described in paragraph (c) of this section. Final pH=7.1±0.1.

(5) *Diluting fluid E.* Dispense 100-milliliter portions of isopropyl myristate into 250-milliliter flasks and sterilize by filtration through a 0.22 micron membrane filter and aseptically dispense into sterile 250-milliliter flasks.

(6) *Diluting fluid F*—To each liter of diluting fluid A add 20 grams of disodium edetate, and adjust with NaOH so that after sterilization the final pH will be 7.1±0.1. Dispense in flasks and sterilize as described in paragraph (c) of this section.

(e) *Conduct of test*—(1) *Bacterial membrane filter method*—(i) *Sample preparation*—(a) *Antibiotic drug.* From each of 20 immediate containers, aseptically transfer approximately 300 milligrams of solids if it is not a liquid drug, or 1 milliliter by volume if it is a liquid drug, or the entire contents if the container contains less than these amounts; except that if it is a liquid drug containing penicillin in a concentration greater than 300,000 units per milliliter, use the

volume that contains 300,000 units, into a sterile 500-milliliter Erlenmeyer flask containing approximately 200 milliliters of diluting fluid A. (If it is a composite sample packaged in one immediate container in accordance with the requirements of § 431.5(b) of this chapter, transfer the entire contents, or approximately 6 grams, into the Erlenmeyer flask.) Stopper the flask and swirl to dissolve the drug. As soon as the sample has completely dissolved, proceed as directed in paragraph (e)(1)(ii) of this section. If the pooled portions from 20 containers will not dissolve completely in 200 milliliters of diluting fluid or will not filter rapidly, 400 milliliters of diluting fluid may be used or two separate tests may be performed using a pool of 10 containers for each test.

(b) *Diluent packaged in combination with a sterile drug.* Using the entire contents from each of 20 immediate containers, proceed as directed in paragraph (e)(1)(ii) of this section.

(c) *Sterile droppers packaged in combination with a sterile drug.* Prepare 20 clean, empty containers of approximately the same size as those in which the sterile antibiotic drug is packaged. To each container add diluting fluid A in a volume approximately the same as that of the sterile drug when it is prepared for dispensing. Cap the containers, sterilize by autoclaving at 121° C. for 20 minutes, and then allow to cool to room temperature. Aseptically open each dropper package and remove each dropper in turn. Use each aseptically to remove 1 milliliter of the fluid from a separate sterile container prepared as described above. Aseptically transfer the fluid to a 500-milliliter Erlenmeyer flask containing approximately 200 milliliters of diluting fluid A. Stopper the flask and proceed as directed in paragraph (e)(1)(ii) of this section.

(ii) *Test procedure.* Aseptically filter the solution through a bacteriological membrane filter. All air entering the filtering system is filtered through air filters capable of removing microorganisms. Filter three 100-milliliter quantities of diluting fluid A through the membrane. For the penicillin and cephalosporin classes of antibiotics, add sufficient penicillinase to diluting fluid A to inactivate the residual antibiotic activity on the membrane after filtration. By means of a sterile circular blade, paper punch, or any other suitable sterile device, cut a circular portion (approximately 17.5 millimeters in diameter) from the center of the filtering area. Transfer the cut center area to a sterile 38 by 200 millimeter (outside dimensions) test tube containing 90±10 milliliters of sterile medium A. Incubate the tube for 7 days at 30°–32° C. Using sterile forceps, transfer the remaining outer portion of the membrane into a second similar tube containing 90±10 milliliters of medium E. Incubate the second tube for 7 days at 22°–25° C.

(2) *Direct method.* From each of 20 immediate containers, transfer approximately 300 milligrams of solids if it is not a liquid drug, or 1 milliliter by volume if it is a liquid drug, or the entire contents if it contains less than these amounts, except if it is a liquid drug containing penicillin in a concentration greater than 300,000 units per milliliter use that volume that contains 300,000 units, into individual sterile test tubes (38 millimeters x 200 millimeters) containing 90±10 milliliters of medium A. Incubate all tubes at 30° C. to 32° C. for 7 days. Gently agitate the tubes every 1 to 3 days or until complete solubilization occurs. At intervals, examine all tubes for visible growth. If growth is observed in any tube, confirm by microscopic examination. From each of the same 20 immediate containers, transfer a second portion (equivalent to that portion initially transferred to the tubes containing medium A) to individual sterile test tubes (38 millimeters x 200 millimeters) containing 90±10 milliliters of medium E, except when each container does not have sufficient material to provide for the two similar-size portions, obtain the second portion from 20 additional immediate containers. Incubate all tubes at 22° C. to 25° C. for 7 days. Gently agitate the tubes every 1 to 3 days or until complete solubilization occurs. At intervals, examine all tubes for visible growth. If growth is observed in any tube, confirm by microscopic examination.

(3) *Bacterial membrane filter method for ophthalmic ointments*—(i) *Ointments that do not contain penicillin.* From each of 10 immediate containers aseptically transfer 0.1 gram of the product into a sterile 250-milliliter flask containing 100 milliliters of diluting fluid E which has previously been heated to a temperature of 47° C. Repeat the process, using 10 additional containers. Swirl both of the flasks to dissolve the ointment. Immediately aseptically filter each solution through a separate bacteriological membrane filter previously moistened with approximately 0.2 milliliter of medium K. Filter all air entering the system through air filters capable of removing microorganisms. Remove any residual antibiotic from the membranes by rinsing each filter five times with 100 milliliters of medium K. The membranes should be covered with fluid throughout each step of the filtration procedure until the end of the last filtering step. By means of a sterile circular blade, paper punch, or other suitable sterile device, cut a circular portion (approximately 17.5 millimeters in diameter) from the center of the filtering area of each membrane. Transfer the center portion of the filtering area of each filter to a sterile test tube 38 millimeters x 200 millimeters (outside dimensions) containing 90 milliliters±10 milliliters of sterile medium I. Incubate the tube for 7 days at 30° C. to 32° C. Using sterile forceps transfer the outer portion of each filter to a similar test tube containing 90 milliliters±10 milliliters of sterile medium J. Incubate this tube for 7 days at 22° C. to 25° C.

(ii) *Ointments containing penicillin.* Proceed as directed in paragraph (e)(3)(i) of this section, except in lieu of sterile medium I use sterile medium L for the center portion of the filtering area of each filter and in lieu of sterile medium J use sterile medium M for the remaining outer portion of each filter.

(f) *Evaluation of results*—(1) *Bacterial membrane-filter method.* The batch, or the part of the batch represented by a particular filling operation, meets the requirements of the test if no sample tube shows growth. If growth is observed in any sample tube, run a second test in the appropriate medium, except perform it in duplicate, using 40 immediate containers. If in the original test, growth is observed in only one of the two media, test both portions of the cut filter membrane by placing each into a separate tube of the same medium. The batch meets the requirements if no tube on the second test shows growth. If growth is observed in any of the control tubes as well as in the sample tubes in either the original or the second test, such test is invalid and must be performed again. In any event, further tests may be justified if there is sufficient reason to believe that the results obtained in the first and second tests may not be valid. In such instances, the batch is satisfactory if on the final test no tube shows growth.

(2) *Direct method.* The batch, or the part of the batch represented by a particular filling operation, meets the requirements of the test if no tube shows growth after incubation. If growth is observed in any sample tube, run a second test in the appropriate medium using 40 immediate containers. The batch is satisfactory if, on the second test, no tube shows growth. If growth is observed in any of the control tubes (except inoculated tubes, if the sample is penicillin) as well as in the sample tubes in either the original or the second test, such test is invalid and must be performed again. In any event, further tests may be justified if there is sufficient reason to believe that the results obtained on the first and second tests may not be valid. In such instances the batch is satisfactory if in the final test no tube shows growth.

Subpart C—Biological Test Methods

§ 436.31 Equipment and diluents for use in biological testing.

(a) *Equipment*—(1) *Temperature-measuring devices.* Use an accurate clinical theromometer or any other temperature-measuring device of equal sensitivity that has been tested to determine the time necessary to reach the maximum reading.

(2) *Pyrogen-free glassware.* Render all glassware free from pyrogens by heating at 250° C. for not less than 30 minutes or by any other suitable method.

(3) *Pyrogen-free syringes and needles.* Render all syringes and needles free from pyrogens by heating at 250° C. for not less than 30 minutes or by any other suitable method.

(4) *Pyrogen-free sodium chloride.* Heat sodium chloride for not less than 2 hours at 200° C.

(b) *Diluents.* (1) Diluent 1 (pyrogen-free water): Prepare pyrogen-free water by collecting freshly distilled water and sterilizing it in an autoclave at 121° C.

for not less than 20 minutes. Pyrogen-free water meets the requirements for the absence of pyrogens as described in § 436.32(a)(3) when 10 milliliters per kilogram are administered as described in § 436.32(a)(2). In testing water for the absence of pyrogens, the aliquot to be tested is made isotonic by the addition of pyrogen-free sodium chloride.

(2) Diluent 2 (pyrogen-free saline solution): Prepare an isotonic solution of sodium chloride by dissolving 9.0 grams of pyrogen-free sodium chloride (prepared as described in § 436.31(a)(4)) in pyrogen-free, distilled water (diluent 1) to make 1,000 milliliters. Sterilize in an autoclave at 121° C. for not less than 20 minutes. Pyrogen-free saline solution meets the requirements for the absence of pyrogens as described in § 436.32(a)(3) when 10 milliliters per kilogram are administered as described in § 436.32(a)(2).

(3) Diluent 3 (sterile distilled water): Prepare freshly distilled water. Sterilize in an autoclave at 121° C. for 20 minutes.

(4) Diluent 4 (sterile saline solution): Dissolve 9.0 grams of sodium chloride in distilled water to make 1,000 milliliters. Sterilize in an autoclave at 121° C. for 20 minutes.

(5) Diluent 5 (10 percent gum acacia): Dissolve 10 grams of gum acacia in approximately 50 milliliters of distilled water. Allow to stand overnight at room temperature and dilute to 100 milliliters with distilled water. Filter through cotton. Store under refrigeration.

(6) Diluent 6 (0.5 percent gum acacia in distilled water).

(7) Diluent 7 (1.0N hydrochloric acid).

(8) Diluent 8 (0.1N hydrochloric acid).

(9) Diluent 9 (0.05N sodium hydroxide).

(10) Diluent 10 (1 percent U.S.P. methylcellulose (4,000 centipoises) solution): Dissolve 1 gram of U.S.P. methylcellulose (4,000 centipoises) in 100 milliliters of distilled water. Allow to stand overnight at room temperature or until solution is complete. Store under refrigeration.

(11) Diluent 11 (0.12*N* sodium hydroxide).

(12) Diluent 12 (0.5 percent methylcellulose (4,000 centipoises) in distilled water). Proceed as directed in paragraph (b)(10) of this section, except use 0.5 gram of methylcellulose (4,000 centipoises).

§ 436.32 Pyrogen test.

(a) *Method 1*—(1) *Test animal.* Use healthy, mature rabbits weighing not less than 1,800 grams each that have maintained their weight on an antibiotic-free diet for at least 1 week under the environmental conditions specified in this section. House the animals individually in an area of uniform temperature (±3° C.) and free from disturbances likely to excite them. Do not use animals for pyrogen tests more frequently than once every 48 hours or prior to 2 weeks following their having been given a test sample that was adjudged pyrogenic. Before using an animal that has not been used for a test during the previous 2 weeks, condition it 1 to 3 days prior to pyrogen testing by conducting a sham test as directed in paragraph (a)(2) of this section, omitting the injection.

(2) *Procedure.* Using equipment and diluents described in § 436.31, as necessary, perform the test in an area where the animals are housed or under similar environmental conditions. On the day of the test: Withhold all food from the animals being used until after completion of the test, except that access to water may be allowed; and determine the "control temperature" of each animal by inserting the temperature-measuring device into the rectum of the test animal to a depth of not less than 7.5 centimeters and allowing sufficient time to reach a maximum temperature, as previously determined, before taking the reading. In any one test use only those animals whose control temperatures do not deviate by more than 1° C. from each other and do not use any animal with a temperature exceeding 39.8° C. The control temperature recorded for each rabbit constitutes the temperature from which any subsequent rise following the injection of the material is calculated. If the product is packaged for dispensing and is in a combination package with a container of diluent, dilute the product as directed in the labeling. Warm the product to be tested to approximately 37° C. Dilute the sample with sterile, pyrogen-free saline (prepared as described in § 436.31(b)(2)) to the appropriate concentration specified in the individual section for each antibiotic to be tested. Inject a test dose of 1 milliliter of the diluted sample per kilogram of rabbit weight into an ear vein of each of three rabbits within 30 minutes subsequent to the control temperature reading. Record the temperature at 1, 2, and 3 hours subsequent to the injection.

(3) *Evaluation.* If no rabbit shows an individual rise in temperature of 0.6° C. or more above its respective control temperature, and if the sum of the three temperature rises does not exceed 1.4° C., the sample meets the requirements for the absence of pyrogens. If one or two rabbits show at temperature rise of 0.6° C. or more, or if the sum of the temperature rises exceeds 1.4° C., repeat the test using five other rabbits. If not more than three of the eight rabbits show individuals rises in temperature of 0.6° C. or more, and if the sum of the eight temperature rises does not exceed 3.7° C., the sample meets the requirements for the absence of pyrogens.

(b) *Method 2.* Proceed as directed in paragraph (a) of this section, except dilute the sample with pyrogen-free water (diluent 1).

(c) *Method 3.* Proceed as directed in paragraph (a) of this section, except dilute the sample with pyrogen-free water (diluent 1) and inject a test dose of 2.0 milliliters of the diluted sample per kilogram of rabbit weight.

(d) *Method 4.* Proceed as directed in paragraph (a) of this section, except inject a test dose of 0.5 milliliter of the diluted sample per kilogram of rabbit weight.

(e) *Method 5.* Proceed as directed in paragraph (a) of this section, except dilute the sample with pyrogen-free water (diluent 1) and inject a test dose of 0.5 milliliter of the diluted sample per kilogram of rabbit weight.

(f) *Method 6.* Proceed as directed in paragraph (a) of this section, except dilute sample with 0.05 *N* sodium hydroxide (diluent 9).

§ 436.33 Safety test.

(a) *Test animal.* Use healthy white mice (preferably of a known strain) not previously used in drug safety testing that are maintained on a diet of pellet food and water ad lib. On the day of test, use only those mice that weight not less than 18 grams nor more than 25 grams. During the test, each group of mice administered a sample should be housed in a suitable container and receive adequate food and water. A constant temperature environment is desirable at all times.

(b) *Preparation and administration of sample solutions.* For each antibiotic drug listed in the table in this paragraph, select the appropriate diluent, test dose (concentration and volume), and route of administration and proceed as directed in paragraph (c) of this section. If the product is packaged for dispensing and is in a combination package with a diluent, dilute the product as directed in the labeling, and then proceed as directed in this paragraph.

Antibiotic drug	Diluent (diluent number as listed in sec. 436.31)	Test dose		Route of administration as described in paragraph (c) of this section
		Concentration in units or milligrams of activity per milliliter	Volume in milliliters to be administered to each mouse	
Aluminum penicillin	3	4,000 units	.5	Intravenous.
Amphotericin B	6	50 mg.	.4	Oral.
Amphotericin B for injection	3	0.05 mg.	.3	Intravenous
Ampicillin	9	20 mg.	.5	Do.
Ampicillin trihydrate	9	20 mg.	.5	Do.
Bacitracin	4	200 units	.5	Do.
Bacitracin methylene disalicylate	5	1,000 units	1.0	Oral.
Benzathine penicillin G	4	4,000 units	.25	Intravenous.
Benzathine phenoxymethyl penicillin	4	4,000 units	.25	Do.
Calcium chlortetracycline syrup	5	25 mg.	.5	Oral.
Calcium novobiocin	4	2 mg.	.5	Intravenous.
Calcium oxytetracycline	5	25 mg.	.5	Oral.
Capreomycin sulfate	4	3.0 mg.	.5	Intravenous.
Carbenicillin indanyl sodium	3	100	.5	Oral
Cefazolin sodium	3	50 mg.	.5	Intravenous.
Cephalexin monohydrate [1]	12	10 mg.	.1	Oral.
Cephaloglycin [1]	5	120.0 mg.	.5	Do.

See footnotes at end of table.

Antibiotic drug	Diluent (diluent number as listed in sec. 436.31)	Test dose: Concentration in units or milligrams of activity per milliliter	Test dose: Volume in milliliters to be administered to each mouse	Route of administration as described in paragraph (c) of this section
Cephaloridine	3	50 mg	.5	Intravenous.
Cephapirin sodium	3	40 mg	.5	Do.
Chloramphenicol	4	5 mg	.5	Do.
Chloramphenicol palmitate	5	60 mg	1.0	Oral.
Chloramphenicol sodium succinate	4	20 mg	.5	Intravenous.
Chloroprocaine penicillin O	4	2,000 units	.5	Do.
Chlortetracycline	(2)	2 mg	.5	Intravenous.
Chlortetracycline bisulfate	3	2 mg	.4	Do.
Chlortetracycline hydrochloride	3	2 mg	.5	Do.
Clemizole penicillin G	(2)	4 mg	.25	Do.
Clindamycin hydrochloride hydrate	4	4 mg	.5	Do.
Clindamycin palmitate hydrochloride	5	50 mg	1.0	Oral.
Clindamycin phosphate	4	20 mg	.5	Intravenous.
Colistin sulfate	5	0.6 mg	.5	Oral.
Cycloserine	4	32 mg	.5	Intravenous.
Demeclocycline	(2)	2 mg	.5	Do.
Demeclocycline hydrochloride	3	2 mg	.5	Do.
Diethylaminoethyl ester penicillin G hydriodide	4	2,000 units	.5	Do.
Dihydrostreptomycin sulfate	3	2 mg	.5	Do.
Disodium carbenicillin	3	40 mg	.5	Do.
Doxycycline hyclate	4	5 mg	.5	Do.
Doxycycline hyclate for injection	4	2 mg	.5	Do.
Doxycycline monohydrate	(2)	100 mg	.5	Oral.
Erythromycin	5	30 mg	1.0	Do.
Erythromycin estolate	5	40 mg	.5	Do.
Erythromycin ethylcarbonate	5	100 mg	.5	Do.
Erythromycin ethylsuccinate	3	80 mg	.5	Do.
Erythromycin gluceptate	3	2 mg	.5	Intravenous.
Erythromycin lactobionate for injection	3	3 mg	.5	Do.
Erythromycin stearate	(2)	80 mg	.5	Oral.
Gentamicin sulfate	4	1 mg	.5	Intravenous.
Gramicidin [3]	5	5 mg	.5	Subcutaneous.
Griseofulvin	5	200 mg	.5	Oral.
Hetacillin	11	45 mg [3]	.4	Intravenous.
Hydrabamine penicillin G [3]	(2)	100 mg	.5	Oral.
Hydrabamine phenoxymethyl penicillin [3]	(2)	100 mg	.5	Do.
Kanamycin sulfate	3	2 mg	.5	Intravenous.
Lincomycin hydrochloride monohydrate	4	4 mg	.5	Do.
Methacycline hydrochloride	3	2 mg	.5	Do.
Minocycline hydrochloride	3	2 mg	.5	Do.
Mitomycin [3]	3	0.2 mg	.5	Do.
Neomycin sulfate	4	0.2 mg	.5	Do.
Nystatin	6	1,200 units	.5	Intraperitoneal.
Oleandomycin phosphate	4	8 mg	.5	Intravenous.
Oxytetracycline	(2)	2 mg	.5	Do.
Oxytetracycline hydrochloride	3	2 mg	.5	Do.
Paromomycin sulfate	3	8 mg	.5	Oral.
Penicillamine	10	100 mg	1.0	Do.
Phenoxymethyl penicillin	4	2,000 units	.5	Intravenous.
Polymyxin B sulfate	4	1,200 units	.5	Do.
Potassium hetacillin	4	8 mg [3]	.5	Do.
Potassium penicillin G	3	4,000 units	.5	Do.
Potassium phenethicillin	4	4,000 units	.5	Do.
Potassium phenoxymethyl penicillin	4	2,000 units	.5	Do.
Procaine penicillin G	4	2,000 units	.5	Do.
Rifampin	10	6 mg	1.0	Oral.
Rolitetracycline	3	2 mg	.5	Intravenous.
Rolitetracycline nitrate	3	2 mg	.5	Do.
Sodium ampicillin	3	40 mg	.5	Do.
Sodium cephalothin	3	40 mg	.5	Do.
Sodium cloxacillin monohydrate	3	16 mg	.5	Do.
Sodium colistimethate	3	1.5 mg	.5	Do.
Sodium dicloxacillin monohydrate	4	20 mg	.5	Do.
Sodium methicillin	4	100 mg	.5	Do.
Sodium nafcillin	4	16 mg	.5	Do.
Sodium nafcillin monohydrate	4	16 mg	.5	Do.
Sodium novobiocin	4	4 mg	.5	Do.
Sodium oxacillin	4	20 mg	.5	Do.
Sodium penicillin G	3	4,000 units	.5	Do.
Spectinomycin hydrochloride	4	16 mg	.5	Do.
Streptomycin sulfate	3	2 mg	.5	Do.
Streptonicozid sulfate	3	1 mg	.5	Do.
Tetracycline	(2)	2 mg	.5	Do.
Tetracycline hydrochloride	3	2 mg	.5	Do.
Tetracycline phosphate	(2)	2 mg	.5	Do.
Troleandomycin	4	200 mg	.5	Oral.
Vancomycin hydrochloride	4	4 mg	.5	Intravenous.
Viomycin sulfate	4	2 mg	.5	Do.
Zinc bacitracin	3	4,000 units	.5	Oral.

[1] To prepare the sample solution, proceed as directed in the individual section of the antibiotic drug regulations in this chapter for the antibiotic to be tested.

[2] Ampicillin activity.

[3] Use the observation period specified in the individual section of the antibiotic drug regulations in this chapter for the antibiotic to be tested in lieu of the 48-hour-observation period specified in paragraph (d) of this section.

(c) *Procedure.* Use sterile glassware, syringes, and needles. To each of five mice, administer the appropriate test dose by one of the following routes of administration:

(1) *Intravenous.* Use a 26-gauge needle, three-quarter inch or 1 inch in length. Inject the test dose into a lateral tail vein of each mouse. The injection should be made at the rate of 0.1 milliliter per second.

(2) *Intraperitoneal.* Use a 26-gauge needle, one-quarter inch in length. Inject the test dose through the abdominal wall into the peritoneal cavity of each mouse.

(3) *Subcutaneous.* Use a 26-gauge needle, one-half inch in length. Inject the test dose subcutaneously at a site on the abdominal or dorsal surface.

(4) *Oral.* By means of a cannula or other suitable device, administer the test dose orally.

(d) *Evaluation.* Observe the mice for 48 hours. Note mortality at 24 hours and 48 hours. If no animal dies within the observation period, the sample passes the safety test. If one or more animals die within the observation period, repeat the test one or more time using for each test 5 or more previously unused mice weighing 20 grams (±0.5 gram) each. If repeat test are required, the sample passes the safety test if the total number of dead mice is no greater than 10 percent of the total number of animals tested, including the original test.

§ 436.35 Histamine test.

(a) *Test animal.* Use healthy, adult cats, each weighing not less than approximately 2.5 kilograms. Either males or nonpregnant females may be used.

(b) *Preparation of the histamine standard.* Use the U.S.P. Reference Standard histamine dihydrochloride. Prepare a solution in sterile distilled water (diluent 3) to contain the equivalent of 1.0 milligram of histamine base per milliliter. Dispense in approximately 0.5-milliliter aliquots in sealed glass ampoules and store under refrigeration until used. Dilute one of the 0.5-milliliter aliquots in 49.5 milliliters of sterile, distilled water (diluent 3) to give a stock solution containing 10 micrograms of histamine base per milliliter. The stock solution may be stored under refrigeration for 1 month. On the day of the test, prepare a standard solution containing 1.0 microgram of histamine base per milliliter of distilled water (diluent 3) and proceed as directed in paragraph (d) of this section.

(c) *Preparation of sample test solution.* For each antibiotic listed in the table in this paragraph, select the appropriate diluent and test dose (concentration and volume) and proceed as directed in paragraph (d) of this section. If the product is packaged for dispensing and is in a combination package with a container of diluent, dilute the product as directed in the labeling.

Antibiotic	Diluent (diluent number as listed in Sec. 436.21(b))	Concentration of test solution (milligrams of activity per milliliter)	Volume of test solution to be injected (milliliters per kilogram of body weight)
Capreomycin sulfate	4	5.0	1.0
Chloramphenicol [1]	4	5.0	0.6
Chloramphenicol sodium succinate	4	5.0	.6
Chlortetracycline hydrochloride	3	5.0	.6
Clindamycin phosphate	4	5.0	1.0
Dihydrostreptomycin sulfate	4	5.0	1.0
Doxycycline hyclate	4	5.0	1.0
Lincomycin hydrochloride monohydrate	4	5.0	1.0
Mino cycline hydrochloride	4	5.0	.6
Mithramycin	3	0.050	1.0
Mitomycin	4	0.050	1.0
Oxytetracycline [1]	3	5.0	.6
Oxytetracycline hydrochloride	4	5.0	.6
Rolitetracycline	4	5.0	.6
Rolitetracycline nitrate	4	5.0	.6
Sodium colistimethate	4	5.0	1.0
Spectinomycin hydrochloride	4	15.0	1.0
Streptomycin sulfate	4	3.0	1.0
Tetracycline hydrochloride	4	5.0	.6
Tetracycline phosphate [1]	3	5.0	.6
Viomycin sulfate	4	5.0	.6

[1] To prepare the test solution, proceed as directed in the individual section of the antibiotic drug regulation in this chapter for the antibiotic to be tested.

(d) *Procedure.* Determine the weight of the animal and place under general anesthesia by intraperitoneal injection of any suitable medium- or long-acting anesthetic that does not unduly affect the level or stability of the blood pressure. Surgically expose the common carotid artery and by blunt dissection separate it completely from all surrounding structures, including the vagus nerve. Insert a cannula connected to a manometer arranged for making a continuous record of blood pressure. Surgically expose the femoral vein. Start the recording kymograph and inspect the tracings for amplitude of excursion and relative stability of pressure. Determine the sensitivity of the animal by injecting into the femoral vein standard solutions of histamine made to contain the equivalent of 1.0 microgram of histamine base per milliliter (prepared as described in paragraph (b) of this section). Make injections at not less than 5-minute intervals using doses of 0.05, 0.1, and 0.15 microgram of histamine base per kilogram of animal weight. Repeat these injections, disregarding the first series of readings, until the drop given by equivalent doses of histamine is relatively uniform. The decrease in blood pressure given by 0.1 microgram per kilogram of histamine base (not less than 20 millimeters of mercury) is subsequently employed as the standard in testing samples. Prepare the test solution by diluting the sample to the proper concentration with the appropriate diluent as designated in paragraph (c) of this section. Inject the appropriate dose of the test solution alternating with a dose of the standard solution equivalent to 0.1 microgram of histamine base per kilogram, maintaining the 5-minute injection schedule. (When a common cannula is used for both the standard and sample solutions, each injection of the standard and test solution should be immediately followed by an injection of approximately 2.0 milliliters of sterile saline (described in § 436.31(b)(4)) to flush any residual activity from the tubing.) If a significant decrease in blood pressure is encountered, the test dose is repeated after the animal has been retested with the standard histamine. The animal may be used as long at it remains reasonably stable and responsive to histamine.

(e) *Evaluation of results.* The product is satisfactory if the decrease in blood pressure obtained with the appropriate dose of the test solution is not greater than the decrease obtained with 0.1 microgram of histamine base per kilogram of body weight.

Subpart D—Microbiological Assay Methods

§ 436.100 Laboratory equipment.

Equipment should be selected which is adequate for its intended use and should be thoroughly cleansed after each use to remove any antibiotic residues. The equipment should be kept covered when not in use. Clean glassware intended for holding and transferring the test organisms should be sterilized in a hot air oven at 200–220° C. for 2 hours. Volumetric flasks, pipettes, or accurately calibrated diluting devices should be used when diluting standard and sample solutions.

(a) *Microbiological agar diffusion assay*—(1) *Cylinders.* Use stainless steel cylinders with an outside diameter of 8 millimeters (±0.1 millimeter), an inside diameter of 6 millimeters (±0.1 millimeter), and a length of 10 millimeters (±0.1 millimeter).

(2) *Plates.* Plastic or glass Petri dishes may be used, having dimensions of 20 by 100 millimeters. Covers should be of porcelain, glazed on the outside. These covers should be washed frequently enough to keep them clean, heated in a muffle furnace, and cooled before use.

(b) *Microbiological turbidimetric assay*—(1) *Tubes.* Tubes which give satisfactory results and have uniform length and diameter should be used. If reusable tubes are employed, care must be taken to remove not only all antibiotic residues from the previous test but also all traces of cleaning solution.

(2) *Colorimeter.* Use a suitable photoelectric colorimeter at a wavelength of 530 millimicrons. Set the instrument at zero absorbance with clear, uninoculated broth prepared as described in the applicable method for the antibiotic being assayed.

§ 436.101 Solutions.

(a) Antibiotic assay solutions are prepared as follows (solution numbers 1, 2, 3, 4, and 6 correspond to those used in "Assay Methods of Antibiotics," D. C. Grove and W. A. Randall, Medical Encyclopedia, Inc., New York, N.Y. (1955)[2] p. 222):

(1) *Solution 1 (1 percent potassium phosphate buffer, pH 6.0).*

Dibasic potassium phosphate: 2.0 gm.
Monobasic potassium phosphate: 8.0 gm.
Distilled water, q.s: 1,000.0 ml.

Adjust with 18*N* phosphoric acid or 10*N* potassium hydroxide to yield a pH 5.95 to 6.05 after sterilization.

(2) *Solution 2 (citrate buffer solution, pH 6.3).*

Citric acid: 13.2 gm.
Sodium hydroxide: 7.06 gm.
Sodium citrate: 97.0 gm.
Distilled water, q.s: 1,000.0 ml.

Adjust with 10 percent citric acid solution or 10*N* sodium hydroxide to yield pH 6.2 to 6.4 after sterilization.

(3) *Solution 3 (0.1M potassium phosphate buffer, pH 8.0).*

Dibasic potassium phosphate: 16.73 gm.
Monobasic potassium phosphate: 0.523 gm.
Distilled water, q.s: 1,000.0 ml.

Adjust with 18*N* phosphoric acid or 10*N* potassium hydroxide to yield a pH 7.9 to 8.1 after sterilization.

(4) *Solution 4 (0.1M potassium phosphate buffer, pH 4.5).*

Monobasic potassium phosphate: 13.6 gm.
Distilled water, q.s: 1,000.0 ml.

Adjust with 18*N* phosphoric acid or 10*N* potassium hydroxide to yield a pH 4.45 to 4.55 after sterilization.

(5) [Reserved]

(6) *Solution 6 (10 percent potassium phosphate buffer, pH 6.0).*

Dibasic potassium phosphate: 20.0 gm.
Monobasic potassium phosphate: 80.0 gm.
Distilled water, q.s: 1,000.0 ml.

Adjust with 18*N* phosphoric acid or 10*N* potassium hydroxide to yield a pH 5.95 to 6.05 after sterilization.

(7) through (9) [Reserved]

(10) *Solution 10 (0.2M potassium phosphate buffer, pH 10.5).*

Dibasic potassium phosphate: 35.0 gm.
10 N potassium hydroxide: 2.0 ml.
Distilled water, q.s: 1,000.0 ml.

Adjust with 18*N* phosphoric acid or 10*N* potassium hydroxide to yield a pH 10.4 to 10.6 after sterilization.

(11) *Solution 11 (10 percent potassium phosphate buffer, pH 2.5).*

Monobasic potassium phosphate: 100.0 gm.
Concentrated hydrochloric acid: 0.2 ml. (approximately).
Distilled water, q.s: 1,000.0 ml.

Adjust with 18*N* phosphoric acid or 10*N* potassium hydroxide to yield a pH 2.0 to 2.8 after sterilization.

[2] Available from: Medical Encyclopedia Inc., 30 East 60th St., New York, NY

(12) *Solution 12 (10 percent potassium phosphate buffer, pH 7.0).*

Monobasic potassium phosphate: 100.0 gm.
Distilled water, q.s: 1,000.0 ml.

Adjust with 18*N* phosphoric acid or 10*N* potassium hydroxide to yield a pH 6.95 to 7.05 after sterilization.

(13) *Solution 13 (0.01N methanolic hydrochloric acid).*

1.0N hydrochloric acid: 10.0 ml.
Methyl alcohol, q.s: 1,000.0 ml.

(14) *Solution 14 (2 percent sodium bicarbonate solution).*

Sodium bicarbonate: 20.0 gm.
Distilled water, q.s: 1,000.0 ml.

Prepare daily.

(15) *Solution 15 (80 percent isopropyl alcohol solution).*

Isopropyl alcohol: 800.0 ml.
Distilled water, q.s: 1,000.0 ml.

(16) [Reserved]

(17) *Solution 17 (5 percent methyl alcohol in 1 percent potassium phosphate buffer, pH 6.0).*

Methyl alcohol: 50.0 ml.
1 percent potassium phosphate buffer, pH 6.0, q.s.: 1,000.0 ml.

§ 436.102 Culture media.

(a) *Ingredients.* Use ingredients that conform to the standards, if any, prescribed by the U.S.P. or N.F. In lieu of preparing the media from the individual ingredients specified, they may be made from dehydrated mixtures that, when reconstituted with distilled water, have the same composition as such media. Minor modifications of the individual ingredients specified in this section are permissible if the resulting media possess growth-promoting properties at least equal to the media described.

(b) *Description of media.* Medium numbers 1, 2, 3, 4, 5, 8, 9, 10, 11, and 13 correspond to those used in "Assay Methods of Antibiotics," D. C. Grove and W. A. Randall, Medical Encyclopedia, Inc., New York, N.Y. (1955)[2] p. 220. Medium numbers 18 through 21 correspond to those used in "Outline of Details for Official Microbiological Assays of Antibiotics," A. Kirshbaum and B. Arret, "Journal of Pharmaceutical Sciences," vol. 56, No. 4, April 1967,[3] p. 512.

(1) *Medium 1.*

Peptone: 6.0 gm.
Pancreatic digest of casein: 4.0 gm.
Yeast extract: 3.0 gm.
Beef extract: 1.5 gm.
Dextrose: 1.0 gm.
Agar: 15.0 gm.
Distilled water, q.s: 1,000.0 ml.
pH 6.5 to 6.6 after sterilization.

(2) *Medium 2.*

Peptone: 6.0 gm.
Yeast extract: 3.0 gm.
Beef extract: 1.5 gm.
Agar: 15.0 gm.
Distilled water, q.s: 1,000.0 ml.
pH 6.5 to 6.6 after sterilization.

(3) *Medium 3.*

Peptone: 5.0 gm.
Yeast extract: 1.5 gm.
Beef extract: 1.5 gm.
Sodium chloride: 3.5 gm.
Dextrose: 1.0 gm.
Dipotassium phosphate: 3.68 gm.
Potassium dihydrogen phosphate: 1.32 gm.
Distilled water, q.s: 1,000.0 ml.
pH 6.95 to 7.05 after sterilization.

(4) *Medium 4.*

Peptone: 6.0 gm.
Yeast extract: 3.0 gm.
Beef extract: 1.5 gm.
Dextrose: 1.0 gm.
Agar: 15.0 gm.
Distilled water, q.s: 1,000.0 ml.
pH 6.5 to 6.6 after sterilization.

(5) *Medium 5.* Medium 5 is the same as medium 2, except adjust the final pH to 7.8 to 8.0 after sterilization.

(6) and (7) [Reserved]

(8) *Medium 8.* Medium 8 is the same as medium 2, except adjust the final pH to 5.8 to 6.0 after sterilization.

(9) *Medium 9.*

Pancreatic digest of casein: 17.0 gm.
Papaic digest of soybean: 3.0 gm.
Sodium chloride: 5.0 gm.
Dipotassium phosphate: 2.5 gm.
Dextrose: 2.5 gm.
Agar: 20.0 gm.
Distilled water q.s: 1,000.0 ml.
pH 7.2 to 7.3 after sterilization.

(10) *Medium 10.* Medium 10 is the same as medium 9, except:

Agar: 12.0 gm.
Polysorbate 80 (add polysorbate 80 after boiling the medium to dissolve the agar): 10.0 ml.
pH7.2 to 7.3 after sterilization.

(11) *Medium 11.* Medium 11 is the same as medium 1, except adjust the final pH to 7.8 to 8.0 after sterilization.

(12) [Reserved]

(13) *Medium 13.*

Peptone: 10.0 gm.
Dextrose: 20.0 gm.
Distilled water, q.s: 1,000.0 ml.
pH 5.6 to 5.7 after sterilization.

(14) through (17) [Reserved]

(18) *Medium 18.* Medium 18 is the same as medium 11, except boil to dissolve the ingredients and then add 20 milliliters of polysorbate 80.

(19) *Medium 19.*

Peptone: 9.4 gm.
Yeast extract: 4.7 gm.
Beef extract: 2.4 gm.
Sodium chloride: 10.0 gm.
Dextrose: 10.0 gm.
Agar: 23.5 gm.
Distilled water, q.s: 1,000.0 ml.
pH 6.0 to 6.2 after sterilization.

(20) *Medium 20.*

Dextrose: 40.0 gm.
Peptone: 10.0 gm.
Agar: 15.0 gm.
Chloramphenicol: 0.05 gm. (activity)
Distilled water, q.s: 1,000.0 ml.
pH 5.6 to 5.7 after sterilization.

(21) *Medium 21.* Use medium 20, sterilized and cooled to 50° C. Aseptically add 2 milliliters of a sterile cyclohexamide solution to each 100 milliliters of the melted agar. Sterile cyclohexamide solution is prepared by filtering a solution containing 10 milligrams of cyclohexamide per milliliter of distilled water through a membrane filter of 0.22-micron porosity.

(22) *Medium 22.*

Dextrose: 40.0 gm.
Peptone: 10.0 gm.
Distilled water, q.s.: 1,000.0 ml.
pH 5.6 to 5.7 after sterilization.

(23) *Medium 23.* Use medium 1, sterilized and cooled to 50° C. Aseptically add sufficient sterile erythromycin solution to give a final concentration of 600 micrograms of erythromycin activity per milliliter of medium. Sterile erythromycin solution is prepared by filtering a solution containing 10 milligrams of erythromycin per milliliter of distilled water through a membrane filter of 0.22-micron porosity.

(24) *Medium 24.* Same as medium 1, except use 30 grams of agar per liter, in lieu of 15 grams per liter.

(25) *Medium 25.* Use medium 1, sterilized and cooled to 50° C. Aseptically add sufficient sterile dihydrostreptomycin sulfate solution to give a final concentration of 1,000 micrograms of dihydrostreptomycin activity per milliliter of medium. Sterile dihydrostreptomycin sulfate solution is prepared by filtering a solution containing 10 milligrams of dihydrostreptomycin per milliliter of distilled water through a membrane filter of 0.22-micron porosity.

(26) *Medium 26.* Use medium 1, sterilized and cooled to 50° C. Aseptically add sufficient sterile tetracycline hydrochloride solution to give a final concentration of 100 micrograms of tetracycline activity per milliliter of medium. Sterile tetracycline hydrochloride solution is prepared by filtering a solution containing 10 milligrams of tetracycline per milliliter of distilled water through a membrane filter of 0.22-micron porosity.

(27) *Medium 27.* Use medium 1, sterilized and cooled to 50° C. Aseptically add sufficient sterile neomycin sulfate solution to give a final concentration of 100 micrograms of neomycin activity per milliliter of medium. Sterile neomycin sulfate solution is prepared by filtering a solution containing 10 milligrams of neomycin per milliliter of distilled water through a membrane filter of 0.22-micron porosity.

(28) *Medium 28.* Use medium 1, sterilized and cooled to 50° C. Aseptically add sufficient sterile dihydrostreptomycin sulfate solution to give a final concentration of 500 micrograms of dihydrostreptomycin activity per milliliter of medium. Sterile dihydrostreptomycin sulfate solution is prepared by filtering a solution containing 10 milligrams of dihydrostreptomycin per milliliter of distilled water through a membrane filter of 0.22-micron porosity.

(29) *Medium 29.*

Beef extract: 6.0 gm.
Peptone: 10.0 gm.
Agar: 15.0 gm.
Distilled water, q.s.: 1,000.0 ml.
pH 7.8 to 8.0 after sterilization.

(30) and (31) [Reserved]

(32) *Medium 32.* Prepare as medium 1, except add 300 milligrams of hydrated manganese sulfate ($MnSO_4 \cdot H_2O$) to each liter of medium.

[2] Available from: Medical Encyclopedia Inc., 30 East 60th St., New York, NY.

[3] Available from: American Pharmaceutical Association, 2215 Constitution Ave N.W., Washington, DC 20037.

(33) *Medium 33.* Use medium 1, sterilized and cooled to 50° C. Aseptically add sufficient sterile sodium novobiocin solution to give a final concentration of 10 micrograms of novobiocin activity per milliliter of medium. Sterile sodium novobiocin solution is prepared by filtering a solution containing 2.5 milligrams of novobiocin per milliliter of distilled water through a membrane filter of 0.22-micron porosity.

§ 436.103 Test organisms.

(a) *Preparation of test organism suspensions.* For each test organism listed in the following table, select the media (as listed by medium number in § 436.102 (b)), incubation period of the Roux bottle, suggested dilution factor, and suggested storage period for the particular test organism and proceed by the appropriate method described in paragraph (b) of this section. Test organism letters A through K, M and N correspond to those used in "Outline of Details for Official Microbiological Assays of Antibiotics," A. Kirshbaum and B. Arret, "Journal of Pharmaceutical Sciences," vol. 56, No. 4, p. 512 (Apr. 1967).[3]

Test organisms	Method used	Medium used for the—		Incubation period of Roux bottle	Suggested dilution factor	Suggested storage period of suspensions under refrigeration
		Slants	Roux bottles			
Test organism A—*Staphylococcus aureus* (ATCC 6538P).[2]	1	1	1	24 hours	1:20	1 week.
Test organism B—*Sarcina subflava* (ATCC 7468).[2]	1	1	1	24 hours	1:30	2 weeks.
Test organism C—*Sarcina lutea* (ATCC 9341)[2]	1	1	1	24 hours	1:40	2 weeks.
Test organism D—*Staphylococcus epidermidis* (ATCC 12228).[2]	1	1	1	24 hours	[1]1:14	1 week.
Test organism E—*Saccharomyces cerevisiae* (ATCC 9763).[2]	6 or 7	19	19	48 hours	1:80	4 weeks.
Test organism F—*Bordetella bronchiseptica* (ATCC 4617).[2]	1	1	1	24 hours	1:20	4 weeks.
Test organism G—*Bacillus cereus* var. *mycoides* (ATCC 11778).[2]	3	1	1	1 week	1:20	2 weeks.
Test organism H—*Bacillus subtilis* (ATCC 6633).[2]	1 or 2	1	1	24 hours	1:20	6 months.
Test organism I—*Klebsiella pneumoniae* (ATCC 10031).[2]	1	1	33	5 days	1:25	6 months.
Test organism J—*Escherichia coli* (ATCC 10536).[2]	1	1	1	24 hours	1:20	1 week.
Test organism K—*Streptococcus faecalis* (ATCC 10541).[2]	1	1	1	24 hours	1:20	2 weeks.
Test organism L—*Micrococcus flavus* (ATCC 10240).[2]	5					24 hours.
Test organism M—*Microsporum gypseum* (ATCC 14683).[2]	1	1	1	24 hours	1:25	4 weeks.
Test organism N—*Sarcina lutea*, resistant to dihydrostreptomycin (ATCC 9341a).[2]	4					2 months.
Test organism O—*Staphylococcus aureus*, resistant to novobiocin (ATCC 12692).[2]	1	1	1	24 hours	1:40	2 weeks.
Test organism P—*Staphylococcus aureus*, resistant to dihydrostreptomycin (ATCC 6538–DR).	1	33	33	24 hours	1:10	4 weeks.
Test organism Q—*Staphylococcus aureus*, resistant to tetracycline (ATCC 12715).[2]	1	25	25	24 hours	1:20	4 weeks.
Test organism R—*Sarcina subflava*, resistant to dihydrostreptomycin (ATCC 7468/d).[2]	1	26	26	24 hours	1:20	1 week.
Test organism S—*Sarcina lutea*, resistant to erythromycin (ATCC 18967).[2]	1	28	28	24 hours	1:20	2 weeks.
Test organism T—*Saccharomyces cerevisiae* (ATCC 2601).[2]	1	23	23	24 hours	1:40	2 weeks.
Test organism U—*Micrococcus flavus*, resistant to neomycin (ATCC 14452).[2]	7	19	19	48 hours	1:20	4 weeks.
Test organism V—*Micrococcus flavus*, resistant to dihydrostreptomycin (ATCC 10240A).[2]	1	27	27	48 hours	1:35	4 weeks.
Test organism W—*Pseudomonas aeruginosa* (ATCC 25619).[2]	1	26	26	48 hours	1:35	4 weeks.
	1	1	1	24 hours	1:25	2 weeks.

[1] If the antibiotic to be tested is oleandomycin, paromomycin, or triacetyloleandomycin, the dilution factor is 1:35.
[2] Available from American Type Culture Collection, 12301 Parklawn Dr., Rockville, MD. 20852.

(b) *Methods for preparation of test organism suspensions*—(1) *Method 1*—(i) *Preparation of suspension.* Maintain organisms on agar slants containing 10 milliliters of the appropriate medium. Incubate the slants at 32° C.–35° C. for 24 hours. Using 3 milliliters of sterile U.S.P. saline T.S., wash the growth from the agar slant onto a large agar surface, such as a Roux bottle, containing 250 milliliters of the appropriate medium. Spread the suspension of organisms over the entire surface of the Roux bottle with the aid of sterile glass beads. Incubate the Roux bottle at 32° C.–35° C. Wash the resulting growth from the agar surface with 50 milliliters of sterile U.S.P. saline T.S.

(ii) *Standardization of suspension.* Determine the dilution factor that will give a 25-percent light transmission at a wavelength of 580 millimicrons using a suitable photoelectric colorimeter and a 13-millimeter diameter test tube as an absorption cell. It may be necessary to adjust the suspension. Determine the amount of suspension to be added to each 100 milliliters of agar or nutrient broth by the use of test plates or test broth. Store the test organism suspension under refrigeration.

(2) *Method 2.* Proceed as directed in paragraph (b)(1) of this section, except in lieu of paragraph (b)(1)(ii) thereof, heat-shock and standardize the suspension as follows: Centrifuge and decant the supernatant liquid. Resuspend the sediment with 50 to 70 milliliters of sterile U.S.P saline T.S. and heat the suspension for 30 minutes at 70° C. Use test plates to assure the viability of the spores and to determine the amount of spore suspension to be added to each 100 milliliters of agar. Maintain the spore suspension under refrigeration.

(3) *Method 3.* Proceed as directed in paragraph (b)(1) of this section, except in lieu of paragraph (b)(1)(ii) thereof, heat-shock and standardize the suspension as follows: Heat the suspension for 30 minutes at 70° C. Wash the spore suspension three times with 25 to 50 milliliters of sterile distilled water. Resuspend the organisms in 50 to 70 milliliters of sterile distilled water and heat-shock again for 30 minutes at 70° C. Use test plates to assure the viability of the spores and to determine the amount of spore suspension to be added to each 100 milliliters of agar. Maintain the spore suspension under refrigeration.

(4) *Method 4.* Grow the organisms for 6 to 8 weeks at 25° C. in several 3-liter wide-mouth Erlenmeyer flasks, each containing 200 milliliters of Medium 22 as described in § 436.102(b)(22). Check the growth for sporulation. When sporulation is 80 percent or greater, harvest the spores on the mycelial layer with a sterile spatula or other convenient instrument. The spores are on top of the floating mat. Place harvested spore material in 50 milliliters of saline.

(5) *Method 5.* Maintain the test organisms in 100-milliliter quantities of nutrient broth—Medium 3 as described in § 436.102(b)(3). For the test prepare a fresh subculture by transferring a loopful of the stock culture to 100 milliliters of the same nutrient broth and incubate for 16 to 18 hours at 37° C. Store this broth culture under refrigeration.

(6) *Method 6.* Maintain the test organisms on agar slants containing 10 milliliters of the medium specified in paragraph (a) of this section. Incubate the slants at 32° C.–35° C. for 24 hours. Inoculate 100 milliliters of nutrient broth—Medium 13 as described in § 436.102(b)(13). Incubate for 16 to 18 hours at 37° C. Proceed as directed in paragraph (b)(1)(ii) of this section.

(7) *Method 7.* Proceed as directed in paragraph (b)(1) of this section, except incubate the slants at 30° C. for 24 hours and incubate the Roux bottle at 30° C. for 48 hours.

§ 436.104 Penicillin activity.

Use penicillin-free equipment and glassware.

(a) *Preparation of inoculated plates.* Proceed as directed in § 436.105(a), using 10 milliliters of medium 1 for the base layer. For the seed layer, use 4 milliliters of medium 4, inoculated with the amount of test organism C which gave the clearest, sharpest zones of inhibition measuring 17 to 21 millimeters in diameter when standardized as described in § 436.103(b)(1)(ii). Disposable plastic covers may be used in lieu of porcelain covers. Use the plates the same day they are prepared.

(b) *Preparation of working standard stock solutions and standard response*

[3] Available from: American Pharmaceutical Association, 2215 Constitution Ave N.W., Washington, DC 20037.

lines solutions. Proceed as directed for penicillin G in § 436.105(b), except dilute the working standard stock solution to a final concentration of 100 units of penicillin G per milliliter and use the following final concentrations for the standard response line: 0.005, 0.0125, 0.025, 0.050, 0.100, and 0.200 unit of penicillin G per milliliter. The 0.050 unit of penicillin G-per-milliliter solution is the reference concentration of the assay.

(c) *Sample preparation.* Dissolve 1.0 gram of the sample in sufficient distilled water to make 18 milliliters. Filter if not clear. Transfer 9.0 milliliters to a separatory funnel, and add 20 milliliters of amyl acetate. Add 1 milliliter of 10 percent potassium phosphate buffer, pH 2.5 (solution 11 as described in § 436.101), shake, allow to separate, and draw off the aqueous layer into a second separatory funnel. Check the pH of the aqueous solution with pH paper, and readjust with concentrated hydrochloric acid if the pH is three or above. Extract again with 20 milliliters or amyl acetate, discard the aqueous phase, and combine the amyl acetate extracts. Wash the extracts with 10 milliliters of 1 percent potassium phosphate buffer, pH 2.5, and discard the buffer wash. Extract the penicillin from the amyl acetate with a 10-milliliter aliquot of 1 percent potassium phosphate buffer, pH 6.0 (solution 1 as described in § 436.101). This is the assay solution.

(d) *Procedure for assay.* For the standard response line, use a total of 15 plates, (three plates for each response line solution, except the reference concentration solution, which is included on each plate). On each set of three plates, fill three alternate cylinders with the reference concentration solution and the other three cylinders with the concentration of the response line under test. Thus, there will be 45 reference concentration zones of inhibition and nine zones of inhibition for each of the other concentrations of the response line. Treat a portion of the sample solution (2 to 5 milliliters) with 0.1 milliliter of penicillinase solution and incubate at 37° C. for 1 hour. For each sample tested, use three plates. On each plate fill two cylinders with the 0.050 unit of penicillin G per milliliter standard, two cylinders with the untreated sample, and two cylinders with the penicillinase-treated sample. Incubate all plates, including those of the standard response line, overnight at 30° C. A zone of inhibition with the untreated sample and no zone with the penicillinase-treated sample are a positive test for penicillin. If a position test is obtained, measure the diameters of the zones of inhibition using an appropriate measuring device such as a millimeter rule, calipers, or an optical projector.

(e) *Estimation of penicillin G activity.* To prepare the standard response line, average the diameters of the standard reference concentration and average the diameters of the standard response line concentration tested for each set of three plates. Average also all 45 diameters of the reference concentration. The average of the 45 diameters of the reference concentration is the correction point of the response line. Correct the average diameter obtained for each concentration to the figure it would be if the average reference concentration diameter for that set of three plates were the same as the correction point. Thus, if in correcting the 0.025 penicillin G concentration, the average of the 45 readings of the 0.050 unit of penicillin G-per-milliliter concentration is 18.5 millimeters and the average of the 0.050 unit of penicillin G-per milliliter concentration of this set of three plates is 18.3 millimeters, the correction is +0.2 millimeters. If the average reading of the 0.025 unit of penicillin G-per-milliliter concentration of these same three plates is 15.5 millimeters, the corrected value is 15.7 millimeters. Plot these corrected values, including the average of the 0.050 unit of penicillin G-per-milliliter concentration, on semilogarithmic graph paper using the penicillin concentration in units per milliliter on the logarithmic scale and the diameter of the zone of inhibition on the arithmetic scale. Draw the line of best fit through these points. To estimate the sample potency, average the zone diameters of the standard and the zone diameters of the sample on the three plates used. If the average zone diameter of the sample is lower than that of the standard, substract the difference between them from the reference concentration diameter of the standard response line. From the response line, read the concentrations corresponding to these corrected values of zone diameters. Multiply the concentration by the dilution factor to obtain the units of penicillin G per sample size tested.

§ 436.105 Microbiological agar diffusion assay.

Using the sample solution prepared as described in the section for the particular antibiotic to be tested, proceed as described in paragraphs (a), (b), (c), and (d) of this section.

(a) *Preparation of inoculated plates.* For each antibiotic listed in the table in this paragraph, select the media (as listed by medium number in § 436.102 (b)), the amount of media to be used in the base and seed layers, the test organism (as listed in § 436.103(a)), and the suggested inoculum and prepare the inoculated plates as follows: Prepare the base layer by adding the appropriate amount of agar to each Petri dish (20 by 100 millimeters). Distribute the agar evenly in the plates and allow it to harden on a flat, level surface. To prepare the seed layer, add the suggested inoculum of the test organism suspension to a sufficient amount of agar, which has been melted and cooled to 48° C.–50° C. Swirl the flask to obtain a homogeneous suspension, and add the appropriate amount of the inoculated media to each of the plates containing the uninoculated base agar. Spread evenly over the agar surface, cover with porcelain covers glazed on the outside, and allow to harden on a flat, level surface. After the agar has hardened, place 6 cylinders described in § 436.100(a)(1) on the inoculated agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius.

Antibiotic	Media to be used (as listed by medium number in § 436.102(b))		Milliliters of media to be used in the base and seed layers		Test Organism	Suggested volume of standardized inoculum to be added to each 100 milliliters of seed agar	Incubation Temperature for the plates
	Base layer	Seed layer	Base layer	Seed layer			
						Milliliters	*Degrees C.*
Amphomycin	2	1	21	4	U	0.5	37
Amphotericin B	None	10	None	8	E	1.0	30
Ampicillin	11	11	21	4	C	0.5	32–35
Bacitracin	2	1	21	4	B	0.2	32–35
Bacitracin	2	1	21	4	L	0.2	32–35
Carbenicillin	9	10	21	4	W	[3] 0.5	37
Cefazolin	2	1	21	4	A	0.05	32–35
Cephalexin	2	1	21	4	A	0.05	32–35
Cephaloglycin	2	1	21	4	A	0.2	32–35
Cephaloridine	2	1	21	4	A	0.1	32–35
Cephalothin	2	1	21	4	A	0.1	32–35
Cephapirin	2	1	21	4	A	0.08	32–35
Chloramphenicol	1	1	21	4	C	2.0	32–35
Chlortetracycline	8	8	21	4	G	[1]	30
Clindamycin	11	11	21	4	C	1.5	37
Cloxacillin	2	1	21	4	A	0.1	32–35
Colistimethate, sodium	9	10	21	4	F	0.1	37
Colistin	9	10	21	4	F	0.1	37
Cycloserine	2	1	10	4	A	0.04	30
Dactinomycin	5	5	10	4	H	[1]	37
Dicloxacillin	2	1	21	4	A	0.1	32–35
Dihydrostreptomycin	5	5	21	4	H	[1]	37
Erythromycin	11	11	21	4	C	1.5	32–34
Gentamicin	17	11	31	4	D	0.02	37
Griseofulvin	20	21	6	4	M	[1]	30 (for 48 hours)
Kanamycin B	5	5	21	4	H	[1]	37
Lincomycin	11	11	21	4	C	1.5	32–34
Methicillin	2	1	21	4	A	0.2	32–35
Mithramycin	8	8	10	4	A	0.1	32–34
Mitomycin	8	8	10	4	H	0.5	37
Nafcillin	2	1	21	4	A	0.2	32–35
Neomycin	11	11	21	4	A	0.4	32–35
Neomycin	11	11	21	4	D	1.0	37

See footnotes at end of table.

Antibiotic	Media to be used (as listed by medium number in § 436.102(b))		Milliliters of media to be used in the base and seed layers		Test Organism	Suggested volume of standardized inoculum to be added to each 100 milliliters of seed agar	Incubation Temperature for the plates
	Base layer	Seed layer	Base layer	Seed layer			
						Milliliters	*Degrees C.*
Novobiocin	2	1	21	4	D	4.0	35
Nystatin	None	19	None	8	T	1.0	30
Oleandomycin	11	11	21	4	D	1.0	37
Oxacillin	2	1	21	4	A	0.2	32-35
Oxytetracycline	8	8	21	4	G	(1)	30
Paromomycin	11	11	21	4	D	2.0	37
Penicillin G	2	1	21	4	A	1.0	32-35
Phenethicillin	11	11	21	4	C	0.5	32-35
Phenoxymethyl penicillin	2	1	21	4	A	1.0	32-35
Polymyxin B	0	10	21	4	F	0.1	37
Rifampin	2	2	21	4	H	0.1	30
Streptomycin	5	5	21	4	H	(2)	37
Tetracycline	8	8	21	4	G	(2)	30
Troleandomycin	18	18	21	4	D	1.0	37
Vancomycin	8	8	10	4	H	(1)	37
Viomycin	5	5	10	4	H	(1)	37

[1] Determine the amount of the inoculum by the use of test plates.
[2] Use dilution of the suspension that gives 25-percent light transmission in lieu of the stock suspension.

(b) ***Preparation of working standard stock solutions and standard response line solutions.*** For each antibiotic listed in the table in this paragraph, select the working standard drying conditions, solvent(s), concentrations, and storage time for the standard solutions and proceed as follows: If necessary, dry the working standard as described in § 436.200; dissolve and dilute an accurately weighed portion to the proper concentration to prepare the working standard stock solution. Store the working standard stock solution under refrigeration and do not use longer than the recommended storage time. Further dilute an aliquot of the working standard stock solution to the proper concentrations to prepare the standard response line solutions. The reference concentration of the assay is the mid concentration of the response line.

Antibiotic	Working standard stock solutions					Standard response line concentrations	
	Drying conditions (method number as listed in § 436.200)	Initial solvent	Diluent (solution number as listed in § 436.101(a))	Final concentration units or milligrams per milliliter	Storage time under refrigeration	Diluent	Final concentrations, units or micrograms of antibiotic activity per milliliter
Amphomycin. (The working standard stock solution should be allowed to stand overnight at room temperature to allow complete solution to take place.)	1		3	0.1 mg	14 days	3	6.4, 8.0, 10.0, 12.5, 15.6 μg.
Amphotericin B	1		Dimethylsulfoxide	1 mg.[1]	use same day	10	0.64 0.80, 1.00, 1.25, 1.56 μg. (Prepare the standard response line simultaneously with the sample solution.)
Ampicillin	Not dried		Distilled water	0.1 mg	1 week	3	0.064, 0.080, 0.100, 0.125 0.156 μg. (Prepare the standard response line simultaneously with the sample solution.)
Bacitracin	1		1	100 units	7 days	1	0.64, 0.80, 1.00, 1.25, 1.56 units.
Carbenicillin	Not dried		1	1 mg	2 weeks	1	12.8, 16.0, 20.0, 25.0, 31.2 μg.
Cefazolin	Not dried	10,000 μg per ml. in solution 6.	1	1 mg	5 days	1	0.64, 0.80, 1.00, 1.25, 1.56 μg.
Cephalexin	Not dried		1	1 mg	7 days	1	12.8, 16.0, 20.0, 25.0, 31.2 μg.
Cephaloglycin	Not dried		Distilled water	100 μg	1 week	4	6.4, 8.0, 10.0, 12.5, 15.6 μg.
Cephaloridine	1		1	1 mg	5 days	1	0.64 0.80, 1.00, 1.25, 1.56 μg.
Cephalothin	1		1	1 mg	5 days	1	0.64, 0.80, 1.00, 1.25, 1.56 μg.
Cephapirin	Not dried		1	1 mg	3 days	1	0.64, 0.80, 1.00, 1.25, 1.56 μg.
Chloramphenicol	Not dried	10,000 μg. per ml. in ethyl alcohol.	1	1 mg	1 month	1	32.0, 40.0, 50.0, 62.5, 78.1 μg.
Chlortetracycline	Not dried		0.01*N* HCl	1 mg	4 days	4	0.064, 0.080, 0.100, 0.125, 0.156 μg.
Clindamycin	Not dried		Distilled water	1 mg	1 month	3	0.64, 0.80, 1.00, 1.25 1.56 μg.
Cloxacillin	Not dried		1	1 mg	7 days	1	3.20, 4.00, 5.00, 6.25, 7.81 μg.
Colistimethate, sodium	1	10,000 μg. per ml. in distilled water.	6	1 mg	use same day	6	0.64, 0.80, 1.00, 1.25, 1.56 μg.
Colistin	1	10,000 μg. per ml. in distilled water.	6	1 mg	2 weeks	6	0.64, 0.80, 1.00, 1.25, 1.56 μg.
Cycloserine	1		Distilled water	1 mg	1 month	1	32.0, 40.0, 50.0 62.5, 78.1 μg.
Dactinomycin	1	10,000 μg. per ml. in methyl alcohol.	3	1 mg	3 months	3	0.50, 0.71, 1.00, 1.41, 2.00 μg.
Dicloxacillin	Not dried		1	1 mg	7 days	1	3.20, 4.00, 5.00, 6.25, 7.81 μg.
Dihydrostreptomycin	8		8	1 mg	30 days	3	0.64, 0.80, 1.00, 1.25, 1.56 μg.
Erythromycin	1	10,000 μg. per ml. in methyl alcohol.	3	1 mg	14 days	3	0.64, 0.80, 1.00, 1.25, 1.56 μg.
Gentamicin	3		3	1 mg	1 month	3	0.064, 0.080, 0.100, 0.125, 0.156 μg.
Griseofulvin	Not dried		Dimethylformamide	1 mg [4]	3 months	3	3.20, 4.00, 5.00, 6.25, 7.81 μg.
Kanamycin B (use the kanamycin sulfate working standard).	Not dried		3	1 mg	1 month	3	0.64, 0.80, 1.00, 1.25, 1.56 μg.
Lincomycin	Not dried		Distilled water	1 mg	1 month	3	1.28, 1.60, 2.00, 2.50, 3.12 μg.
Methicillin	Not dried		1	1 mg	4 days	1	6.4, 8.0, 10.0, 12.5, 15.6 μg.
Mithramycin	7		Distilled water	0.1 mg	1 day	1	0.50, 0.7, 1.00, 1.41, 2.00 μg.
Mitomycin	Not dried		1	1 mg	14 days	1	0.50, 0.71, 1.0, 1.41, 2.0 μg.
Nafcillin	Not dried		1	1 mg	2 days	1	1.28, 1.60, 2.00, 2.50, 3.12 μg.

Antibiotic	Working standard stock solutions					Standard response line concentrations	
	Drying conditions (method number as listed in §436.201)	Initial solvent	Diluent (solution number as listed in §436.101 (a))	Final concentration units or milligrams per milliliter	Storage time under refrigeration	Diluent	Final concentrations, units or micrograms of antibiotic activity per milliliter
Neomycin	1		3	1 mg.	2 weeks	3	0.64, 0.80, 1.00, 1.25, 1.56 μg. (if test organism D is used); 6.4, 8.0, 10.0, 12.5, 15.6 μg. (if test organism A is used).
Novobiocin	5	10,000 μg. per ml. in absolute ethyl alcohol.	3	1 mg.	5 days	6	0.320, 0.400, 0.500, 0.625, 0.781 μg.
Nystatin	4		Dimethylformamide.	1,000 units [1]	use same day	6	12.8, 16.0, 20.0, 25.0, 31.2 units. (Prepare the standard response line solutions simultaneously with the sample solution to be tested using red low actinic glassware.)
Oleandomycin	Not dried	10,000 μg. per ml. in ethyl alcohol.	3	1 mg.	30 days	3	3.20, 4.00, 5.00, 6.25, 7.81 μg.
Oxacillin	Not dried		1	1 mg.	3 days	1	3.20, 4.00, 5.00, 6.25, 7.81 μg.
Oxytetracycline	Not dried		0.1*N* HCl	1 mg.	4 days	4	0.64, 0.80, 1.00, 1.25, 1.56 μg.
Parmomycin	1		3	1 mg.	3 weeks	3	0.64, 0.80, 1.00, 1.25, 1.56 μg.
Penicillin G	Not dried		1	1,000 units	2 days	1	0.64, 0.80, 1.00, 1.25, 1.56 units.
L-phenethicillin	Not dried		Distilled water	1,000 units	1 week	3	0.064, 0.080, 0.100, 0.125, 0.156 unit.
Phenoxymethyl penicillin	Not dried	2 ml. of methyl alcohol.	1	100 units	4 days	1	0.64, 0.80, 1.00, 1.25, 1.56 units.
Polymyxin B	1	Distilled water [2]	6	10,000 units	2 weeks	6	6.4, 8.0, 10.0, 12.5, 15.6 units.
Rifampin	Not dried		Methyl alcohol	1 mg.	1 day	1	3.20, 4.00, 5.00, 6.25, 7.81, μg./ml.
Streptomycin	1		3	1 mg.	30 days	3	0.64, 0.80, 1.00, 1.25, 1.56 μg.
Tetracycline	Not dried		0.1*N* HCl	1 mg.	1 day	4	0.64, 0.80, 1.00, 1.25, 1.56 μg.
Vancomycin	1		Distilled water	0.4 mg.	1 week	4	6.4, 8.0, 10.0, 12.5, 15.6 μg.
Viomycin	1		Distilled water	1 mg.	1 week	3	32.0, 40.0, 50.0, 62.5, 78.1 μg.

[1] Further dilute aliquots of the working standard stock solution with dimethylsulfoxide to give concentrations of 12.8, 16, 20.0, 25, and 31.2 micrograms per milliliter.
[2] Further dilute aliquots of the working standard stock solution with dimethylformamide to give concentrations of 256, 320, 400, 500, and 624 units per milliliter.
[3] Add 2 milliliters of distilled water for each 5 milligrams of weighed working standard material.
[4] Further dilute aliquots of the working standard stock solution with dimethylformamide to give concentrations of 64, 80, 100, 125, and 156 micrograms per milliliter.

(c) *Procedure for assay.* For the standard response line, use a total of 12 plates—three plates for each response line solution, except the reference concentration solution which is included on each plate. On each set of three plates, fill three alternate cylinders with the reference concentration solution and the other three cylinders with the concentration of the response line under test. Thus, there will be 36 reference concentration zones of inhibition and nine zones of inhibition for each of the four other concentrations of the response line. For each sample tested use three plates. Fill three alternate cylinders on each plate with the standard reference concentration solution and the other three cylinders with the sample reference concentration solution. After all the plates have incubated for 16 to 18 hours at the appropriate incubation temperature for each antibiotic listed in the table in paragraph (b) of this section, measure the diameters of the zones of inhibition using an appropriate measuring device such as a millimeter rule, calipers, or an optical projector.

(d) *Estimation of potency.* To prepare the standard response line, average the diameters of the standard reference concentration and average the diameters of the standard response line concentration tested for each set of three plates. Average also all 36 diameters of the reference concentration for all four sets of plates. The average of the 36 diameters of the reference concentration is the correction point of the response line. Correct the average diameter obtained for each concentration to the figure it would be if the average reference concentration diameter for that set of three plates were the same as the correction point. Thus, if in correcting the highest concentration of the response line, the average of the 36 diameters of the reference concentration is 16.5 millimeters and the average of the reference concentration of the set of three plates (the set containing the highest concentration of the response line) is 16.3 millimeters, the correction is +0.2 millimeter. If the average reading of the highest concentration of the response line of these same three plates is 16.9 millimeters, the corrected diameter is then 17.1 millimeters. Plot these corrected diameters, including the average of the 36 diameters of the reference concentration on 2-cycle semilog paper, using the concentration of the antibotic in micrograms or units per milliliter as the ordinate (the logarithmic scale), and the diameter of the zone of inhibition as the abscissa. The response line is drawn either through these points by inspection or through points plotted for highest and lowest zone diameters obtained by means of the following equation:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

L=Calculated zone diameter for the lowest concentration of the standard response line;

H=Calculated zone diameter for the highest concentration of the standard response line;

c=Average zone diameter of 36 readings of the reference point standard solution;

a, b, d, e=Corrected average values for the other standard solutions, lowest to highest concentration, respectively.

To estimate the potency of the sample, average the zone diameters of the standard and the zone diameters of the sample on the three plates used. If the average zone diameter of the sample is larger than that of the standard, add the difference between them to the reference concentration diameter of the standard response line. If the average zone diameter of the sample is lower than that of the standard, subtract the difference between them from the reference concentration diameter of the standard response line. From the response line, read the concentrations corresponding to these corrected values of zone diameters.

Multiply the concentration by the appropriate dilution factor to obtain the antibiotic content of the sample.

§ 436.106 Microbiological turbidimetric assay.

Using the sample solution prepared as described in the section for the particular antibiotic to be tested, proceed as described in paragraphs (a), (b), and (c) of this section.

(a) *Preparation of working standard stock solutions and standard response line solutions.* For each antibiotic listed in the table in this paragraph, select the working standard drying conditions, solvent(s), concentrations, and storage time for the standard solutions and proceed as follows: If necessary, dry the working standard as described in § 436.200; dissolve and dilute an accurately weighed portion to the proper concentration for the working standard stock solution. Store the working standard stock solution under refrigeration and do not use longer than the recommended storage time. Prepare the proper concentrations for the standard response line solutions by further diluting an aliquot of the working standard stock solution. The reference concentration of the assay is the mid concentration of the standard response line.

Antibiotic	Working standard stock solutions					Standard response line concentrations	
	Drying conditions (method number as listed in §436.200	Initial solvent	Diluent (solution number as listed in §436.101 (a))	Final concentration	Storage time under refrigeration	Diluent (solution number as listed in §436.101(a))	Final concentrations—units or micrograms of antibiotic activity per milliliter
Candicidin [1]	6		Dimethyl sulfoxide.	1 mg.	Use same day	Distilled water	0.030, 0.043, 0.060, 0.085, 0.120 μg. (Prepare standard response line simultaneously with the sample solution.)
Capreomycin	5	Distilled water		1 mg.	7 days	Distilled water	64, 80, 100, 125, 156 μg.
Chloramphenicol	Not dried	Ethyl alcohol (10,000 μg. per ml.).	1	1 mg.	1 month	1	2.00, 2.24, 2.50, 2.80, 3.12 μg.
Chlortetracycline	Not dried		0.01*N* HCl	1 mg.	4 days	4	0.038, 0.048, 0.060, 0.075, 0.094 μg.
Cycloserine	1		Distilled water	1 mg.	1 month	Distilled water	32.0, 40.0, 50.0, 62.5, 78.1 μg.
Demeclocycline	1		0.1*N* HCl	1 mg.	4 days	4	0.064, 0.080, 0.100, 0.125, 0.156 μg.
Dihydrostreptomycin	5		Distilled water	1 mg.	30 days	Distilled water	24.0, 26.8, 30.0, 33.5, 37.5 μg.
Doxycycline	Not dried		0.1*N* HCl	1 mg.	5 days	4	0.064, 0.080, 0.100, 0.125, 0.156 μg.
Gramicidin	1		95% ethyl alcohol	1 mg.	30 days	95% ethyl alcohol	0.028, 0.034, 0.040, 0.048, 0.057 μg
Kanamycin	Not dried		Distilled water	1 mg.	1 month	Distilled water	8.0, 8.9, 10.0, 11.2, 12.5 μg.
Methacycline	1		13	1 mg.	7 days	4	0.038, 0.048, 0.060, 0.075, 0.094 μg.
Minocycline	Not dried		0.1*N* HCl	1 mg.	2 days	4	0.079, 0.084, 0.100, 0.120, 0.143 μg.
Oxytetracycline	Not dried		0.1*N* HCl	1 mg.	4 days	4	0.154, 0.192, 0.240, 0.300, 0.375 μg.
Rolitetracycline	1		Methanol	1 mg.	1 day	4	0.160, 0.200, 0.250, 0.312, 0.390 μg.
Spectinomycin	Not dried		Distilled water	1 mg.	1 month	Distilled water	24.0, 26.8, 30.0, 33.5, 37.5 μg
Streptomycin	1		Distilled water	1 mg.	30 days	Distilled water	24.0, 26.8, 30.0, 33.5, 37.5 μg.
Tetracycline	Not dried		0.1*N* HCl	1 mg.	1 day	4	0.154, 0.192, 0.240, 0.300, 0.375 μg.
Troleandomycin	1		15	1 mg.	Use same day	1	16.0, 20.0, 25.0, 31.2, 39.0 μg.
Tyrothricin [2]							
Viomycin	1		Distilled water	1 mg.	7 days	Distilled water	64, 80, 100, 125, 156 μg.

[1] Use sterile equipment for all stages of this assay.
[2] The gramicidin working standard and the gramicidin standard response line concentrations are used for the assay of tyrothricin.

(b) *Procedure for assay.* For each antibiotic listed in the table in this paragraph, select the test organism (as listed in § 436.103(a)), nutrient broth (as listed by medium number in § 436.102(b)), and suggested inoculum and proceed as follows: Place 1.0 milliliter (or 0.1 milliliter in the case of gramicidin and tyrothricin) of each concentration of the standard response line (prepared as described in paragraph (a) of this section) and of the sample solution in each set of three replicate tubes (as described in § 436.100(b)(1)). Fifteen tubes are used for the five-point standard response line and three for each sample. To each tube add 9 milliliters of the inoculated broth and place immediately in a water bath at the appropriate temperature for 2 to 4 hours. The exact length of the incubation period should be determined by observation of growth in the reference concentration tube of the standard. Remove the tubes from the water bath and add 0.5 milliliter of a 12-percent formaldehyde solution to each tube. Determine the absorbance value of each tube in a suitable photoelectric colorimeter, at a wavelength of 530 millimicrons. Set the instrument at zero absorbance with an uninoculated blank composed of the same amounts of nutrient broth and formaldehyde used in the assay.

Antibiotic	Test organism	Medium (nutrient broth)	Suggested volume of standardized inoculum to be added to each 100 milliliters of medium (nutrient broth)	Incubation temperature
			Milliliters	*Degrees C.*
Candicidin [1]	E	13	0.2	[2] 28
Capreomycin	I	3	0.05	37
Chloramphenicol	J	3	0.7	37
Chlortetracycline	A	3	0.1	37
Cycloserine	A	3	0.4	37
Demeclocycline	A	3	0.1	37
Dihydrostreptomycin	I	3	0.1	37
Doxycycline	A	3	0.1	37
Gramicidin	K	3	1.0	37
Kanamycin	A	3	0.2	37
Methacycline	A	3	0.1	37
Minocycline	A	3	0.2	37
Oxytetracycline	A	3	0.1	37
Rolitetracycline	A	3	0.1	37
Spectinomycin	J	3	0.1	37
Streptomycin	I	3	0.1	37
Tetracycline	A	3	0.1	37
Troleandomycin	I	3	0.1	37
Tyrothricin	K	3	1.0	37
Viomycin	I	3	0.1	37

[1] Use sterile equipment for all stages of this assay.
[2] Cover and incubate the tubes for 16 to 18 hours.

$$L=\frac{2a+2b+c-d}{5},$$

$$H=\frac{2c+2d+c-a}{5},$$

where:

L = Calculated absorbance value for the lowest concentration of the standard response line.

H = Calculated absorbance value for the highest concentration of the standard response line.

a, b, c, d, e = Average absorbance values for each concentration of the standard response line, lowest to the highest, respectively.

(c) *Estimation of potency.* To prepare the standard response line, plot the average absorbance values for each concentration of the standard response line on one-cycle semilogarithmic graph paper with the absorbance values on the arithmetric scale and concentrations on the logarithmic scale. The response line is drawn either through these points by inspection or through points plotted for highest and lowest absorbance values obtained by means of the following equations.

To estimate the potency of the sample, average the absorbance values for the sample and determine the antibiotic concentration from the standard response line. Multiply the concentration by the appropriate dilution factor to obtain the antibiotic content of the sample.

Subpart E—General Chemical Tests for Antibiotics

§ 436.200 Loss on drying.

Use the method specified in the individual section for each antibiotic.

(a) *Method 1.* In an atmosphere of about 10 percent relative humidity, grind the sample, if necessary, to obtain a fine powder. When tablets, troches, or capsules are to be tested, use four tablets, troches, or capsules in preparing the sample. Transfer about 100 milligrams of the sample to a tared weighing bottle equipped with a ground-glass stopper. Weigh the bottle and place it in a vacuum oven, tilting the stopper on its side so that there is no closure during the drying period. Dry at a temperature of 60° C. and a pressure of 5 millimeters of mercury or less for 3 hours. At the end of the drying period, fill the vacuum oven with air dried by passing it through a drying agent such as sulfuric acid or silica gel. Replace the stopper and place the weighing bottle in a desiccator over a desiccating agent, such as phosphorous pentoxide or silica gel, allow to cool to room temperature, and reweigh. Calculate the percent of loss.

(b) *Method 2.* Proceed as directed in paragraph (a) of this section, except use a tared weighing bottle or weighing tube equipped with a capillary-tube stopper, the capillary having an inside diameter of 0.20–0.25 millimeter, and place it in a vacuum oven without removing the stopper.

(c) *Method 3.* Proceed as directed in paragraph (a) of this section, except dry the sample at a temperature of 110° C. and a pressure of 5 millimeters of mercury or less for 3 hours.

(d) *Method 4.* Proceed as directed in paragraph (a) of this section, except dry the sample at a temperature of 40° C. and a pressure of 5 millimeters of mercury or less for 2 hours.

(e) *Method 5.* Proceed as directed in paragraph (a) of this section, except dry the sample at a temperature of 100° C. and a pressure of 5 millimeters of mercury or less for 4 hours.

(f) *Method 6.* Proceed as directed in paragraph (a) of this section, except dry the sample at a temperature of 40° C. and a pressure of 5 millimeters of mercury or less for 3 hours.

(g) *Method 7.* Proceed as directed in paragraph (a) of this section, except dry the sample at a temperature of 25° C. and a pressure of 5 millimeters of mercury or less for 4 hours.

§ 436.201 Moisture determination.

(a) *Equipment*—(1) *Apparatus.* Use a closed system consisting of all glass automatic burettes, platinum electrodes, and a magnetic stirrer connected to a suitable electrometric apparatus. This apparatus embodies a simple electrical circuit which serves to pass 5 to 10 microamperes of direct current between a pair of platinum electrodes immersed in the solution to be titrated. At the endpoint of the titration a slight excess of the reagent increases the flow of current to between 50 and 150 microamperes for 30 seconds or longer, depending upon the solution being titrated.

(2) *Titrating vessel.* Use a suitable titrating vessel which has been previously dried at 105° C. and cooled in a desiccator.

(b) *Reagents*—(1) *Karl Fischer reagent.* Dissolve 125 grams of iodine in 170 milliliters of pyridine, add 670 milliliters of methanol and cool. To 100 milliliters of pyridine kept in an ice bath, add sulfur dioxide until the volume reaches 200 milliliters. Slowly add this solution to the cooled iodine-methanol-pyridine mixture and shake well. (A commercially prepared Karl Fischer reagent may be used.) Preserve the reagent in glass-stoppered bottles protected from light and from moisture in the air.

(2) *Methanol solution.* Add sufficient water (usually 2 milligrams per milliliter) to methanol so that each milliliter of the resulting methanol solution is equivalent to about 0.5 milliliter of Karl Fischer reagent.

(3) *Solvents*—(i) *Solvent A.* Methanol:chloroform:carbon tetrachloride (1:2:2 by volume).

(ii) *Solvent B.* Chloroform:carbon tetrachloride (1:1 by volume).

(c) *Standardization of reagents*—(1) *Water equivalence of Karl Fischer reagent.* Standardize the Karl Fischer reagent no longer than 1 hour before use by one of the following methods.

(i) Accurately weigh 25–35 milligrams of water into a dry titration vessel and add 20 milliliters of solvent A. Start the stirrer and titrate to the endpoint by adding measured quantities of Karl Fischer reagent. Calculate the water equivalence of the Karl Fischer reagent as follows:

$$e=\frac{W}{V_T-V_A},$$

where:

e = Water equivalence of the Karl Fischer reagent in terms of milligrams of water per milliliter;

W = Milligrams of water;

V_T = Milliliters of Karl Fischer reagent used;

V_A = Milliliters of Karl Fischer reagent equivalent to the 20 milliliters of solvent A, determined as directed in paragraph (c) (3) of this section.

(ii) Accurately weigh about 25–35 milligrams of water into a dry titration vessel, add an excess of Karl Fischer reagent, start the stirrer, and titrate to the endpoint with methanol solution. Calculate the water equivalence of the Karl Fischer reagent as follows:

$$e=\frac{W}{V_T-V_m\times f},$$

where:

e = Water equivalence of the Karl Fischer reagent in terms of milligrams of water per milliliter;

W = Milligrams of water;

V_T = Milliliters of Karl Fischer reagent used;

V_m = Milliliters of methanol solution used;

f = Milliliters of Karl Fischer reagent equivalent to each milliliter of methanol solution determined as directed in paragraph (c) (2) of this section.

(2) *Karl Fischer reagent equivalence of methanol solution.* Titrate a known volume of Karl Fischer reagent with methanol solution until the endpoint is reached. Calculate the milliliters of Karl Fischer reagent equivalent to each milliliter of methanol solution as follows:

$$f=\frac{V_T}{V_m},$$

where:

f = Milliliters of Karl Fischer reagent equivalent to each milliliter of methanol solution;

V_T = Milliliters of Karl Fischer reagent used;

V_m = Milliliters of methanol solution used.

(3) *Karl Fischer reagent equivalence of solvents.* (i) Solvent A: Use 20 milliliters of solvent A as the sample. Start the stirrer and titrate to the endpoint by adding measured quantities of Karl Fischer reagent.

(ii) Solvent B: Use 10 milliliters of solvent B as the sample. Add an excess of Karl Fischer reagent to the sample and start the stirrer. Titrate to the endpoint with methanol solution.

(iii) Calculate the Karl Fischer reagent equivalence of the solvents as follows:

$$V_A=V_T,$$
$$V_B=V_T-V_m\times f,$$

where:

V_A and V_B = Milliliters of Karl Fischer reagent equivalent to the aliquots used of solvents A and B respectively;

V_T = Milliliters of Karl Fischer reagent used;

V_m = Milliliters of methanol solution used;

f = Milliliters of Karl Fischer reagent equivalent to each milliliter of methanol solution determined as directed in paragraph (c) (2) of this section.

(d) *Sample preparation*—(1) *Powders.* In the case of tablets, grind 4 tablets to a fine powder. If the maximum moisture limit is greater than 1 percent, accurately weigh about 300 milligrams of the sample into a dry titrating vessel. If the maximum moisture limit is less than 1 percent, accurately weigh 1 to 2 grams of the sample. Proceed as directed in paragraph (e) (1) or (2) of this section.

(2) *Ointments and oils.* (i) Transfer about 1 to 2 grams, accurately weighed, into a dry titrating vessel. Proceed as directed in paragraph (e) (1) of this section; or

(ii) Transfer about 1 to 2 grams, accurately weighed, into a dry titrating vessel. Add 10 milliliters of solvent B and proceed as directed in paragraph (e) (2) of this section.

(3) *Aerosols with propellant.* Place the immediate container to be tested in a suitable freezing unit having a temperature of not higher than 0° C. for at least 2 hours. Remove the container from the freezing unit, puncture it, mix the entire contents by swirling. Proceed as directed in paragraph (e) (3) of this section, using an accurately measured 10-milliliter aliquot from the container as the sample and allowing the solution to warm to at least 10° C. before determining the endpoint.

(4) *Hygroscopic powders.* Weigh the immediate container. Using a suitable dry hypodermic needle and syringe, inject 3 milliliters of anhydrous methanol into the container and shake to dissolve the contents. Using the same syringe, remove the withdrawable contents and transfer into the titration vessel. Rinse the syringe and needle by drawing in an additional 3 milliliters of anhydrous methanol. Add the rinsings to the titration vessel. Titrate the solution immediately, proceeding as directed in paragraph (e) (3) of this section. Determine the Karl Fisher equivalent (in milliliters), if any, of the anhydrous methanol by titrating a blank of the same total volume used in preparing the sample and rinsing the syringe and needle. Dry the immediate container and its closure for three hours at 100° C., cool to room temperature in a desiccator, and weigh. Determine the weight of sample tested by subtracting the weight obtained from the original weight of the immediate container.

(e) *Titration procedures and calculations*—(1) *Procedure 1.* Add 20 milliliters of solvent A to the sample. Start the stirrer and titrate to the endpoint by adding measured quantities of Karl Fischer reagent. Determine the percent moisture in the sample as follows:

$$\text{Percent moisture} = \frac{(V_T - V_A) \times e \times 100}{W_s},$$

where:

e = Water equivalence of the Karl Fischer reagent determined as directed in paragraph (c) (1) of this section;

V_T = Milliliters of Karl Fischer reagent used;

V_A = Milliliters of Karl Fischer reagent equivalent to the 20 milliliters of solvent A, determined as directed in paragraph (c) (3) of this section;

W_s = Weight of the sample in milligrams.

(2) *Procedure 2.* Add an excess of Karl Fischer reagent to the sample, start the stirrer, and titrate to the endpoint with methanol solution. Calculate the percent moisture in the sample as follows:

(i) For powders:

$$\text{Percent moisture} = \frac{(V_T - V_m f) \times e \times 100}{W_s},$$

(ii) For oils and ointments:

$$\text{Percent moisture} = \frac{(V_T - V_m f - V_B) \times e \times 100}{W_s},$$

where:

V_T = Milliliters of Karl Fischer reagent used;

V_m = Milliliters of methanol solution used;

f = Milliliters of Karl Fischer reagent equivalent to each milliliter of methanol solution determined as directed in paragraph (c) (2) of this section.

V_B = Milliliters of Karl Fischer reagent equivalent to the 10 milliliters of solvent B determined as directed in paragraph (c) (3) of this section;

e = Water equivalence of the Karl Fischer reagent determined as directed in paragraph (c) (1) of this section;

W_s = Weight of the sample in milligrams.

(3) *Procedure 3.* Add about 20 milliliters of solvent A to a dry titrating vessel and proceed as directed in titration procedure 1 or 2. Disregard the volume of reagents used to determine the endpoint. Promptly introduce an accurately weighed or measured quantity of sample into the titrating vessel and titrate to the endpoint using either titration procedure 1 or 2 without additional solvents. Calculate the percent moisture in the sample as follows:

(i) If titration procedure 1 is used:

$$\text{Percent moisture in weighed samples} = \frac{V_T \times e \times 100}{W_s},$$

$$\text{Percent moisture in aerosols} = \frac{V_T \times e}{\text{Milliliters of sample} \times 10},$$

$$\text{Percent moisture in hygroscopic powders} = \frac{(V_T - V_b) \times e \times 100}{W_s},$$

(ii) If titration procedure 2 is used:

$$\text{Percent moisture in weighed samples} = \frac{(V_T - Vmf) \times e \times 100}{W_s},$$

$$\text{Percent moisture in aerosols} = \frac{(V_T - Vmf) \times e}{\text{Milliliters of sample} \times 10},$$

$$\text{Percent moisture in hygroscopic powders} = \frac{(V_T - Vmf - V_b) \times e \times 100}{W_s},$$

where:

V_T = Milliliters of Karl Fischer reagent used;

V_m = Milliliters of methanol solution used;

f = Milliliters of Karl Fischer reagent equivalent to each milliliter of methanol solution determined as directed in paragraph (c) (2) of this section;

V_b = Karl Fischer equivalent (in milliliters) of the methanol used as a sample solvent;

e = Water equivalence of the Karl Fischer reagent determined as directed in paragraph (c) (1) of this section.

§ 436.202 pH.

(a) *Apparatus.* Use a suitable pH meter equipped with a glass and a calomel electrode.

(b) *Standardization.* Standardize the pH meter with two buffer solutions that differ by at least 2 pH units and of which one is within 2 pH units of the expected pH value of the sample.

(c) *Sample preparation.* If necessary, dilute the sample with carbon dioxide-free distilled water to the concentration specified in the individual section for each antibiotic.

(d) *Test procedure.* Determine the pH of the sample at 25°±2° C.

§ 436.203 Crystallinity.

Use the method specified in the individual section for each antibiotic.

(a) *Method 1.* To prepare the sample for examination, mount a few particles in mineral oil on a clean glass slide. Examine the sample by means of a polarizing microscope. The particles reveal the phenomena of birefringence and extinction positions on revolving the microscope stage.

(b) *Method 2.* Proceed as directed in paragraph (a) of this section, except to prepare the sample for examination, mount a few particles in mineral oil, add 1 drop of ethyl alcohol, and allow to react for about 30 seconds.

§ 436.204 Iodometric assay.

(a) *Reagents.* (1) 0.01*N* Sodium thiosulfate (2.482 grams $Na_2S_2O_3 \cdot 5H_2O$ and 125 milligrams Na_2CO_3 per liter).

(2) 1.0*N* Sodium hydroxide.

(3) 1.2*N* Hydrochloric acid.

(4) 0.01*N* Iodine solution (prepared from 0.1*N* iodine U.S.P.).

(5) Starch iodide paste, T.S. (U.S.P.).

(b) *Preparation of sample and working standard solutions*—(1) *Working standard solutions.* From the following table, select the initial solvent, diluent, and final concentration as listed for each antibiotic working standard. Dissolve and dilute an accurately weighed portion to the specified final concentration and proceed as directed in paragraphs (c) and (d) of this section.

Antibiotic	Initial solvent	Diluent (solution number as listed in § 436.101(a))	Final concentration in units or milligrams of activity per milliliter of standard solution
Ampicillin	None	Distilled water	1.25 milligrams.
Cephalexin	do	do	2 milligrams.
Cephaloridine	None	Distilled water	2 milligrams.
Cephalothin	do	do	Do.
Cephapirin	None	Distilled water	2 milligrams.
Cloxacillin	do	do	1.25 milligrams.
Dicloxacillin	do	do	Do.
Methicillin	do	1	Do.
Nafcillin	do	1	1.25 milligrams.
Oxacillin	do	1	1.25 milligrams.
Penicillin G	do	1	2,000 units.
Penicillin O	do	1	Do.
Phenoxymethyl penicillin	1 milliliters methyl alcohol.	1	Do.
Potassium L-phenethicillin	None	1	Do.

(2) *Bulk antibiotic solutions.* From the following table, select the initial solvent, diluent, and final concentration as listed for each antibiotic. Dissolve an accurately weighed aliquot (approximately 30 to 60 milligrams) of the sample, dilute to the appropriate final concentration, and proceed as directed in paragraphs (c) and (d) of this section.

Antibiotic	Initial solvent	Diluent (solution number as listed in § 436.101(a))	Final concentration in units or milligrams of activity per milliliter of sample
Aluminum penicillin	None	2	2,000 units.
Ampicillin	do	Distilled water	1.25 milligrams.
Ampicillin trihydrate	do	do	Do.
Benzathine penicillin G blank solution	do	do	2,000 units.
Benzathine penicillin G inactivated solution	do	1*N* NaOH	2,000 units; allow to stand in 1*N* NaOH for 15 minutes before assaying.
Benzathine phenoxymethyl penicillin blank solution.	do	Distilled water	2,000 units.
Benzathine phenoxymethyl penicillin inactivated solution.	do	1*N* NaOH	2,000 units; allow to stand in 1*N* NaOH for 15 minutes before assaying.
Buffered crystalline penicillin G	do	1	2,000 units.
Cephalexin	None	Distilled water	2 milligrams.
Cephaloridine	None	Distilled water	2 milligrams.
Cephapirin sodium	None	Distilled water	2 milligrams.
Chloroprocaine penicillin O	2 milliliters methyl alcohol.	1	2,000 units.
Diethylaminoethyl ester penicillin G hydrochloride.	do	1	Do.
Hydrabamine phenoxymethyl penicillin	None	Chloroform	Do.
Phenoxymethyl penicillin	2 milliliters methyl alcohol.	1	Do.
Potassium penicillin G	None	1	2,000 units.
Potassium penicillin O	do	1	Do.
Potassium phenethicillin	do	1	Do.
Potassium phenoxymethyl penicillin	do	1	Do.
Procaine penicillin G	2 milliliters methyl alcohol.	1	Do.
Sodium ampicillin	None	1	1.25 milligrams.
Sodium cephalothin	None	Distilled water	2 milligrams.
Sodium cloxacillin monohydrate	do	Distilled water	1.25 milligrams.
Sodium dicloxacillin monohydrate	do	do	Do.
Sodium methicillin	do	1	Do.
Sodium nafcillin	do	1	1.25 milligrams.
Sodium nafcillin monohydrate	do	1	Do.
Sodium oxacillin	do	1	Do.
Sodium penicillin G	do	1	2,000 units.
Sodium penicillin O	do	1	Do.

(3) *Finished product solutions.* Prepare the sample for assay as directed in the individual section for each antibiotic product to be tested by diluting to the concentration prescribed in the table in paragraph (b)(2) of this section and proceed as described in paragraphs (c) and (d) of this section.

(c) *Inactivated sample and standard solutions.* (1) Transfer 2.0 milliliters each of the sample and the appropriate working standard solutions to glass-stoppered Erlenmeyer flasks.

(2) Add 2.0 milliliters of 1*N* sodium hydroxide, except if the sample has been diluted in 1*N* sodium hydroxide, and allow to stand at room temperature for 15 minutes.

(3) Add 2.0 milliliters of 1.2*N* hydrochloric acid.

(4) Add 10.0 milliliters of 0.01*N* iodine solution, stopper, allow to stand at room temperature for 15 minutes, and proceed as directed in paragraph (e) of this section.

(d) *Blank determination.* Transfer 2.0 milliliters each of the sample and the appropriate working standard solutions to glass-stoppered Erlenmeyer flasks. Add 10.0 milliliters of 0.01*N* iodine solution and immediately proceed as directed in paragraph (e) of this section.

(e) *Titration procedure.* Titrate the excess iodine using 0.01*N* sodium thiosulfate. Toward the end of the titration, add 1 drop of the starch iodide paste. Continue the titration by the addition of 0.01- to 0.02-milliliter portions of 0.01*N* sodium thiosulfate, shaking vigorously after each addition. The endpoint is reached when the blue color of the starch-iodine complex is discharged. Calculate the antibiotic content as described in paragraph (f) of this section.

(f) *Calculations*—(1) *F factor determination.* Using the appropriate working standard for the particular antibiotic to be tested, assay the standard as directed in this section. Calculate the F factor (the units or micrograms of activity equivalent of each milliliter of 0.01*N* sodium thiosulfate consumed) by means of the following formula:

$$F=\frac{W_s\times P}{V_s},$$

where:

W_s=Actual weight in milligrams of standard in the 2.0 milliliters titrated;

P=Potency of the working standard in units or micrograms per milligram;

V_s=Milliliters of sodium thiosulfate used in the working standard blank determination minus the milliliters of sodium thiosulfate used in the titration of the inactivated working standard solution (the difference is the equivalent of the number of milliliters of 0.01*N* idoine absorbed by the inactivated standard).

(2) *Bulk antibiotic.* Calculate the potency of the sample in units or micrograms per milligram by means of the following formula:

Units or micrograms of antibiotic per milligram

$$=\frac{V_s\times F}{W_s},$$

where:

V_s=Milliliters of sodium thiosulfate used in the sample blank determination minus the milliliters of sodium thiosulfate used in the titration of the inactivated sample solution (the difference is the equivalent of the number of milliliters of 0.01*N* iodine absorbed by the inactivated sample);

W_s=Actual weight in milligrams of sample in the 2.0 milliliters titrated.

(3) *Finished products.* Calculate the potency of the sample in units or milligrams by means of the appropriate one of the following formulas:

Units of antibiotic per dose or item

$$=\frac{V_s\times F\times d}{2n},$$

Milligrams of antibiotic per dose or item

$$=\frac{V_s\times F\times d}{n\times 2{,}000},$$

where:

d=Dilution factor for the sample;

n=Number of doses or items in the sample assayed.

§ 436.205 Hydroxylamine colorimetric assay.

(a) *Reagents*—(1) *Hydroxylamine hydrochloride solution.* Dissolve 350 grams of hydroxylamine hydrochloride in sufficient distilled water to make 1 liter.

(2) *Buffer.* Dissolve 173 grams of sodium hydroxide and 20.6 grams of sodium acetate in sufficient distilled water to make 1 liter.

(3) *Neutral hydroxylamine.* Mix 1 volume each of hydroxylamine hydrochloride solution described in paragraph (a)(1) of this section and the buffer described in paragraph (a)(2) of this section. Check the pH and if necessary adjust to pH 7.0±0.1 by adding an additional amount of one of the components. To 1 volume of this neutralized solution add 8 volumes of distilled water and 2 volumes of 95 percent ethanol. This solution should be used for 1 day only.

(4) *Ferric ammonium sulfate.* Dissolve 272 grams of ferric ammonium sulfate in a mixture of 26 milliliters of concentrated sulfuric acid and sufficient distilled water to make 1 liter. This reagent may be used for 1 week when stored in a brown bottle at room temperature.

(b) *Preparation of working standard solutions.* From the following table, select the diluent and final concentration as listed for each antibiotic working standard. Dissolve and dilute an accurately weighed portion to the specified final concentration and proceed as directed in paragraph (d) of this section.

Antibiotic	Diluent (solution number as listed in § 436.101(a))	Final concentration in milligrams per milliliter of standard solution
Ampicillin	Distilled water.	1.25
Cefazolin [1]		1.0
Cephaloridine	Distilled water.	1.0
Cephalothin	do	2.0
Cephapirin	do	1.0
Cloxacillin	1	1.25
Dicloxacillin	Distilled water.	1.25
Methicillin	1	1.25
Nafcillin	1	1.25
Oxacillin	1	1.25
Penicillin G	1	1.25
Phenethicillin	1	1.25
Phenoxymethyl penicillin	17	1.25
Procaine penicillin G	17	2.0

[1] To prepare the working standard solution, proceed as directed in the individual section of the antibiotic drug regulation in this chapter for the antibiotic to be tested

(c) *Preparation of sample solutions.* From the following table, select the diluent and final concentration as listed for each antibiotic. Dissolve an accurately weighed portion of the sample, dilute to the appropriate final concentration, and proceed as directed in paragraph (d) of this section; if the product is packaged for dispensing, dilute an aliquot of the stock solution (prepared as described in the individual monograph) to the appropriate concentration and then proceed as directed in paragraph (d) of this section.

Antibiotic	Diluent (solution number as listed in § 436.101(a))	Final concentration in milligrams per milliliter of sample
Ampicillin	Distilled water.	1.25
Ampicillin trihydrate	do	1.25
Cefazolin sodium	1	1.0
Cephaloridine	Distilled water.	1.0
Cephapirin sodium	do	1.0
Phenoxymethyl penicillin	17	1.25
Potassium penicillin G	1	1.25
Potassium phenethicillin	1	1.25
Potassium phenoxymethyl penicillin.	1	1.25
Procaine penicillin G	17	2.0
Sodium ampicillin	Distilled water.	1.25
Sodium cephalothin	do	2.0
Sodium cloxacillin monohydrate.	1	1.25
Sodium dicloxacillin monohydrate.	Distilled water.	1.25
Sodium methicillin monohydrate.	1	1.25
Sodium nafcillin monohydrate.	1	1.25
Sodium oxacillin monohydrate.	1	1.25
Sodium penicillin G	1	1.25

(d) *Procedure.* Using a volume of from 1 to 2 milliliters of standard or sample solution, add an equal volume of water and mix. Add the following reagents in the specified volumetric proportions with respect to the sample or standard solutions: Add 1.25 volumes of neutral hydroxylamine reagent and allow to react for 5 minutes. Add 1.25 volumes of ferric ammonium sulfate reagent, mix, and after 3 minutes determine the absorbance of the resulting solution at the wavelength of 480 millimicrons, using a suitable spectrophotometer and a reagent blank prepared by treating a volume of water in the same manner as the standard or sample solution. The time elapsed after the addition of the ferric ammonium sulfate reagent and the reading of the absorbance must be precisely the same (within 10 seconds) for each solution. Calculate the potency of the sample in units or micrograms per milligram as follows:

$$\text{Units or micrograms per milligram of sample} = \frac{(A_1)\ (\text{Potency (in units or micrograms per milliliter of standard solution)})}{(A_2)\ (\text{Milligrams of sample per milliliter of sample solution})}$$

A_1 = Absorbance of sample solution.
A_2 = Absorbance of standard solution.

§ 436.206 Test for particulate contamination in ophthalmic ointments.

(a) *Procedure.* Extrude the contents of each of 10 tubes as completely as practicable into separate, clear, glass Petri dishes (60 millimeters in diameter), cover the dishes, and heat to 80° C. to 85° C. for at least 2 hours or until the ointment has melted completely and evenly in the dishes. A higher temperature of 100° C.±2° C. may be used if necessary to allow adequate settling of metal particles. Allow the ointment to cool to room temperature without agitation. Invert each Petri dish on the stage of a suitable microscope adjusted to furnish 30 times magnification and equipped with an eye-piece micrometer disc which has been calibrated at the magnification being used. In addition to the usual source of light, direct an illuminator from above the ointment at a 45° angle. Examine the entire bottom of the Petri dish for metal particles. By varying the intensity of the illuminator from above, such metal particles are recognized by their characteristic reflection of light. Count the total number of metal particles exceeding 50 microns in any single dimension.

(b) *Evaluation.* The batch is acceptable if (1) a total of not more than 50 such particles is found in 10 tubes; and (2) not more than one tube is found to contain more than eight such particles. If the batch fails the above test, repeat the test on 20 additional tubes of ointment. The total number of metal particles exceeding 50 microns in any single dimension from the 30 tubes tested shall not exceed 150, with not more than three tubes containing more than eight such particles.

§ 436.207 Residue on ignition.

Use the method specified in the individual section for each antibiotic.

(a) *Method 1.* Place approximately 1 gram of the sample, accurately weighed, in a tared porcelain crucible and carefully ignite at a low temperature until thoroughly charred. The crucible may be loosely covered with a porcelain lid during the charring. Add 2 milliliters of nitric acid and 5 drops of sulfuric acid to the contents of the crucible and cautiously heat until white fumes are evolved, then ignite, preferably in a muffle furnace, at 500° C. to 600° C. until the carbon is all burned off. Cool the crucible in a desiccator and weigh. From the weight of residue obtained, calculate the sulfated ash content.

(b) *Method 2.* Proceed as directed in paragraph (a) of this section, except use 2 milliliters of sulfuric acid and do not use the nitric acid.

§ 436.208 Heavy metals determination.

(a) *Reagents*—(1) *Ammonia solution.* Prepare an aqueous solution containing not less than 9 grams and not more than 10 grams of ammonia (NH_3) per 100 milliliters.

(2) *6 percent acetic acid.* Dilute 60 milliliters of glacial acetic acid with sufficient water to give a solution of 1,000 milliliters.

(3) *Hydrogen sulfide solution.* Prepare a saturated solution of hydrogen sulfide by passing hydrogen sulfide into cold water for a sufficient time. It is suitable if it produces an immediate copious precipitate when added to an equal volume of 1*N* ferric chloride. Prepare a fresh hydrogen sulfide solution each time a heavy metals test is to be performed.

(4) *Lead nitrate stock solution.* Dissolve 159.8 milligrams of lead nitrate with 100 milliliters of 0.15*N* nitric acid, and dilute with water to a volume of 1,000 milliliters. Prepare and store this solution in glass containers free from soluble lead salts.

(5) *Standard lead solution.* Dilute a 10-milliliter aliquot of the lead nitrate stock solution to 100 milliliters with water. This solution must be freshly prepared each time a heavy metals test is performed. One milliliter of this standard lead solution represents a lead level of 10 parts per million in a 1.0-gram sample or 20 parts per million in a 0.5-gram sample.

(b) *Preparation of the sample.* Use the sulfated ash obtained as described in § 436.207(a). If the heavy metal limit is greater than 30 parts per million, the sulfated ash may be obtained from a 0.5-gram sample. Add 2 milliliters of hydrochloric acid to the sulfated ash and slowly evaporate to dryness on a steam bath. Moisten the residue with 1 drop of hydrochloric acid, add 10 milliliters of hot water, and digest by heating on the

steam bath for 2 minutes. After cooling to room temperature, add ammonia solution dropwise until a pH of 7.2 is reached, then add 2 milliliters of 6 percent acetic acid. Filter the solution, if necessary, and wash the crucible and the filter with about 10 milliliters of water. Combine the washings with the filtrate and dilute to exactly 25 milliliters with water.

(c) *Procedure.* Prepare a series of five standard lead solutions, in increments of 10 parts per million, in which the solution of lowest concentration contains 20 parts of lead per million less than the maximum limit of heavy metals permitted for the sample. Transfer the necessary quantities of standard lead solution described in paragraph (a) (5) of this section directly into metal-free 50-milliliter Nessler tubes of uniform diameter, add 2 milliliters of 6 percent acetic acid to each, and adjust each to a final volume of 25 milliliters with water. Transfer the 25-milliliter solution of the sample described in paragraph (b) of this section to another Nessler tube. Add 10 milliliters of hydrogen sulfide solution to each standard and sample solution, mix well, and allow to stand for 10 minutes. View downward over a white surface; the color of the solution of the sample should be no darker than the standard that contains the lead equivalent of the heavy metals limit of the test.

§ 436.209 Melting range or temperature.

(a) *Apparatus.* Melting range apparatus consists of a glass container for a bath of colorless fluid, a suitable stirring device, an accurate thermometer, and a controlled source of heat. Any apparatus or method of equal accuracy may be used. The accuracy should be checked periodically by use of melting point standards, preferably those that melt near the expected melting range of the product to be tested. The bath fluid is selected with a view to the temperature required, but light paraffin is used generally and certain liquid silicones are well adapted to the higher temperature ranges. The fluid is deep enough to permit immersion of the thermometer to its specified immersion depth so that the bulb is still 2 centimeters above the bottom of the bath.

(b) *Sample preparation.* If necessary, reduce the sample to a fine powder and store it in a desiccator over sulfuric acid for 24 hours. If a method for loss on drying is included in the section for the antibiotic to be tested, a sample dried by that method may be used.

(c) *Test procedure.* Use a capillary glass tube about 10 centimeters long and 0.8 to 1.2 millimeters internal diameter with the wall 0.2 to 0.3 millimeter in thickness. Charge the tube with a sufficient amount of the dry power to form a column 2.5 to 3.5 millimeters high from the sealed end when packed down as closely as possible by moderate tapping on a solid surface. Heat the bath until a temperature 10°±1° C. below the expected melting range is reached, then introduce the charged tube, and heat at a rate of rise of 3°±0.5° C. per minute until melting is completed. The temperature at which the column of the sample is observed to collapse definitely against the side of the tube at any point is defined as the beginning of melting, and the temperature at which the sample becomes liquid throughout is defined as the end of melting.

§ 436.210 Specific rotation.

(a) *Test procedure.* The appropriate solvent test concentration, and polarimeter tube length are specified in the section for each antibiotic to be tested. Accurately weigh the sample to be tested in a glass-stoppered volumetric flask, dissolve in the appropriate solvent, and dilute to the specified test concentration at 25° C. Maintain the solution at 25° C. and transfer to the appropriate polarimeter tube. Determine the angular rotation of both solvent and sample solution in a suitable polarimeter, using a sodium light source or a white light source with a 589.3-millimicron filter. The zero correction is the average of the blank readings and is subtracted from the average observed rotation of the sample solution if the two figures are of the same sign, or is added if they are opposite in sign, to give the corrected angular rotation of the sample solution. The determination must be completed within one-half hour from the time the solution is prepared.

(b) *Calculations.* Determine the specific rotation, $[\alpha]$, by the following formula:

$$[\alpha]_x^t = \frac{100a}{lc}$$

where:

a=The corrected angular rotation of the sample solution in degrees at temperature t using a light source of a wavelength of x millimicrons;

l=The length of the polarimeter tube in decimeters;

c=The concentration of the solution expressed as number of grams of substance in 100 milliliters of solution.

§ 436.211 Identity test by infrared spectrophotometry.

(a) *Apparatus*—(1) *Spectrophotometer.* A suitable spectrophotometer capable of recording the infared absorption spectrum in the 2 to 15 micron range.

(2) *Hydraulic press.* A 30-ton hydraulic press with 12-inch square platens.

(b) *Sample preparation methods.* Use the sample preparation method specified in the individual section for each antibiotic.

(1) *Potassium bromide discs.* Quantities of materials specified are for a 13-millimeter die. Appropriate adjustments should be made in the quantities of materials when dies of other sizes are used. To prepare a 1.0 percent mixture weigh approximately 2 milligrams of the sample and mix thoroughly with 200 milligrams of dried potassium bromide (infrared spectrophotometric quality). For a 0.5 percent potassium bromide mixture, use 1 milligram of sample. For a 0.25 percent potassium bromide mixture, use 0.5 milligram of sample. A mortar and pestle, a ball mill, or other suitable mixing device may be used. Transfer the uniformly milled mixture to the die, evacuate gradually while raising the pressure to 3,000 pounds per square inch until evacuation is complete, then raise the pressure to 16,000 pounds per square inch, and hold that pressure for 2 to 3 minutes. Release the pressure, dismantle the die, and recover the potassium bromide disc. Mount the disc in a suitable holder and proceed as directed in paragraph (c) of this section.

(2) *Mineral oil mull.* Weigh approximately 20 milligrams of the sample into an agate mortar and add 2 drops of mineral oil. Triturate thoroughly with a pestle until a uniform consistency is obtained. Use two rock salt plates as an absorption cell. Place a small drop of the mull in the center of one of the plates. Gently put the other plate on the mull and slowly squeeze the plates together to spread the mull uniformly. Clamp the two plates firmly together in a metal holder. Examine the assembled cell by holding it up to the light. It should appear smooth and free of any air bubbles. Proceed as directed in paragraph (c) of this section.

(3) *1 percent solution.* Prepare a 1 percent solution of the sample in chloroform and use 1.0 millimeter matched absorption cells. Proceed as directed in paragraph (c) of this section.

(c) *Procedure.* Place the sample, prepared as directed in paragraph (b) of this section, in the spectrophotometer. Determine the infrared absorbance spectrum between the wavelengths of 2 to 15 microns. To be suitable the spectrum should have a transmittance of between 20 and 70 percent at most of the wavelengths showing significant absorption. Compare the spectrum to that of an authentic sample of the same antibiotic prepared in an identical manner. To pass the infrared identity test, the absorption spectrum of the sample should compare qualitatively with that of the authentic sample.

§ 436.212 Disintegration test.

(a) *Apparatus*—(1) *Basket-rack assembly.* The basket-rack assembly consists of 6 open-ended glass tubes, each 7.75±0.25 centimeters long and having an inside diameter of approximately 21.5 millimeters and a wall approximately 2 millimeters thick; the tubes are held in a vertical position by two plastic plates, each about 9 centimeters in diameter and 6 millimeters in thickness, with six holes, each about 24 millimeters in diameter, equidistant from the center of the plate and equally spaced from one another. Attached by screws to the undersurface of the lower plate is 10-mesh No. 23 (0.025 inch) W. and M. gauge woven stainless steel wire cloth. The glass tubes and the upper plastic plate are secured in position at the top by means of a stainless steel plate, about 9 centimeters in diameter and 1 millimeter in thickness, having six perforations each about 20 millimeters in diameter, which coincide with those of the upper plastic plate and the upper open ends of the glass tubes. A central shaft about 8 centimeters in length, the upper end of which terminates in an eye through

which a string or wire may be inserted, is attached to the stainless steel plate. The parts of the apparatus are assembled and rigidly held by means of three bolts passing through the two plastic plates and the steel plate. The design of the basket-rack assembly may be varied somewhat provided the specifications for the glass tubes and the screen mesh size are maintained.

(2) *Disks.* Each tube is provided with a slotted and perforated cylindrical disk 9.5±0.15 millimeters thick and 20.7±0.15 millimeters in diameter. The disk is made of a suitable, transparent plastic material having a specific gravity of between 1.18 and 1.20. Five 2-millimeter holes extend between the ends of the cylinder, one of the holes being through the cylinder axis and the others parallel with it and equally spaced on a 6-millimeter radius from it. Equally spaced on the sides of the cylinder are four notches that form V-shaped planes perpendicular to the ends of the cylinder. The dimensions of each notch are such that the openings on the bottom of the cylinder are 1.60 millimeters square and those on the top are 9.5 millimeters wide and 2.55 millimeters deep. All surfaces of the disk are smooth.

(3) *Raising and lowering device.* Use a device for raising and lowering the basket in the immersion fluid at a constant rate between 28 and 32 cycles per minute through a distance of not less than 5 centimeters and not more than 6 centimeters.

(b) *Immersion fluids.* During the performance of the tests all immersion fluids are maintained at a temperature of 37°±2° C. by using a thermostatically controlled water bath.

(1) Distilled water.

(2) Simulated gastric fluid: Dissolve 2.0 grams of sodium chloride and 7.0 milliliters of hydrochloric acid in about 500 milliliters of water. Dissolve 3.2 grams of pepsin in this solution and add sufficient water to make 1,000 milliliters. This solution has a pH of about 1.2.

(3) Simulated intestinal fluid: Dissolved 6.8 grams of monobasic potassium phosphate in 250 milliliters of water, mix and add 190 milliliters of 0.2*N* sodium hydroxide and 400 milliliters of water. Add 10.0 grams of pancreatin, mix, and adjust the resulting solution with 0.2*N* sodium hydroxide to a pH of 7.5±0.1. Dilute to 1,000 milliliters.

(c) *Immersion vessel.* Use a suitable vessel, such as a 1-liter beaker.

(d) *Operation.* Add enough immersion fluid to the immersion vessel so that when the basket-rack assembly is placed on the raising and lowering device at the highest point of the upward stroke, the wire mesh remains at least 2.5 centimeters below the surface of the fluid and descends to not less than 2.5 centimeters from the bottom of the immersion vessel.

(e) *Procedure*—(1) *Uncoated or film-coated tablets.* Place one tablet into each of the six tubes of the basket, add a disk to each tube, and operate the apparatus, using simulated gastric fluid as the immersion fluid. At the end of the time limit specified in the individual section for the particular antibiotic tablet being tested, lift the basket from the fluid and observe the tablets.

(2) *Plain-coated tablets.* Place one tablet in each of the six tubes of the basket, add a disk to each tube, and operate the apparatus, using simulated gastric fluids as the immersion fluid. After 30 minutes, lift the basket from the fluid and observe the tablets. If the tablets have not disintegrated completely, substitute simulated intestinal fluid as the immersion fluid and continue the test for a total period of time (including previous immersion in simulated gastric fluid) equal to the time limit specified in the individual section for the particular antibiotic tablet being tested. Lift the basket and observe the tablets.

(3) *Enteric-coated tablets.* Place one tablet in each of the six tubes of the basket and operate the apparatus, using simulated gastric fluid as the immersion fluid. One hour later, lift the basket from the fluid and observe the tablets. If the tablets show no distinct evidence of dissolution or disintegration, add a disk to each tube and operate the apparatus, using simulated intestinal fluid as the immersion fluid, for a total period of time (including the previous immersion in simulated gastric fluid) equal to the time limit specified in the individual section for the particular antibiotic tablet being tested. Lift the basket and observe the tablets.

(f) *Evaluation.* Complete disintegration is defined as that state in which any residue of the tablet (except fragments of the insoluble coating) remaining on the screen is a soft mass having no palpably firm core. The tablets pass the disintegration test if all of the tablets tested disintegrate completely under the conditions and time specified in the individual section for the antibiotic tablet being tested. If one or two tablets fail to disintegrate completely, repeat the test on 12 additional tablets. The tablets pass the disintegration test if not less than 16 of the total of 18 tablets tested disintegrate completely. Enteric-coated tablets fail the disintegration test if they show any distinct evidence of dissolution or disintegration after 1 hour immersion in simulated gastric fluid.

§ 436.213 Nonaqueous titrations.

(a) *Equipment*—(1) *Apparatus.* Use a closed system consisting of a suitable titrimeter equipped with a potentiometer, an automatic burette, a chart recorder, and a glass calomel combination electrode (with saturated methanolic potassium chloride as the electrolyte).

(2) *Titration vessel.* Use a 100-milliliter tall form beaker without a spout.

(b) *Reagents*—(1) Methyl alcohol, reagent grade, anhydrous.

(2) Dimethylsulfoxide, A.C.S., reagent grade.

(3) Glacial acetic acid, A.C.S., reagent grade.

(4) Lithium methoxide reagent: 0.02*N* lithium methoxide in methyl alcohol, standardized against primary grade benzoic acid.

(5) Perchloric acid reagent: 0.02*N* perchloric acid in glacial acetic acid, standardized against primary grade potassium acid phthalate.

(c) *Preparation of sample solutions.* Select the weight of the sample and the solvent listed for each antibiotic. Transfer the accurately weighed sample to a titration vessel. Add the appropriate solvent, cover, and stir magnetically until the sample is dissolved. Proceed as directed in paragraph (e) of this section, using the procedure or procedures specified in the individual section for each antibiotic.

Antibiotic	Weight in milligrams of sample	Solvent
Ampicillin-acid titration.	100	20 milliliters dimethylsulfoxide and 30 milliliters methyl alcohol.*
Ampicillin-base titration.	100	50 milliliters glacial acetic acid.
Ampicillin sodium-base titration.	80	50 milliliters glacial acetic acid.
Cephaloglycin-base titration.	50	50 milliliters glacial acetic acid.

*The methyl alcohol is added after the sample has dissolved in dimethylsulfoxide.

(d) *Blank determination.* Place the same volume of solvent used to prepare the sample solution into a titration vessel and proceed as directed in paragraph (e) of this section, using the procedure or procedures specified in the individual section for each antibiotic.

(e) *Titration procedures*—(1) *Acid titration.* Equilibrate the electrode by soaking it overnight in the solvent used for preparing the sample solution. Start the magnetic stirrer and titrate the sample solution with the lithium methoxide reagent. Record the change in potential of the solution with the addition of the titrant. Determine the number of milliliters of reagent consumed at neutralization (the inflection point of the titration curve). Calculate the antibiotic content as directed in the individual section.

(2) *Base titration.* Proceed as directed in paragraph (e)(1) of this section, except use the perchloric acid reagent as the titrant and calculate the antibiotic content as directed in the individual section.

Subpart F—Chemical Tests for Specific Antibiotics

§ 436.300 Polarimetric assay of carbenicillin indanyl sodium.

(a) *Equipment.* Polarimeter capable of measuring optical rotatory activity at 365 nanometers: Perkin-Elmer Model 141 or equivalent, with a suitable 1-decimeter polarimeter tube.

(b) *Reagents*—(1) *4-methyl-2-pentanone.* Meets ACS specifications.

(2) *Phosphate-citrate buffer.* Dissolve 61.0 grams of anhydrous disodium phosphate and 11.0 grams of citric acid in 950 milliliters of distilled water. Adjust the pH to 6.0 with 6*N* hydrochloric acid. Dilute to 1,000 milliliters with distilled water.

(c) *Preparation of carbenicillin indanyl sodium sample and working standard solutions.* Accurately weigh approximately 125 milligrams of the carbenicillin indanyl sodium sample or

working standard into a 25-milliliter volumetric flask. Dissolve and dilute to volume with distilled water. Transfer a 5-milliliter aliquot to a 50-milliliter glass-stoppered centrifuge tube. Add 15 milliliters of the phosphate-citrate buffer and 20 milliliters of 4-methyl-2-pentanone; stopper and shake the tube for 10 seconds. Centrifuge at 2,000 revolutions per minute for 10 minutes to separate the phases. Remove about 15 milliliters of the upper (4-methyl-2-pentanone solvent) phase and proceed as directed in paragraph (e) of this section.

(d) *Preparation of the blank.* Place a 5-milliliter aliquot of distilled water into a 50-milliliter glass-stoppered centrifuge tube, add 15 milliliters of phosphate-citrate buffer and 20 milliliters of 4-methyl-2-pentanone; stopper and shake the tube for 10 seconds. Centrifuge at 2,000 revolutions per minute for 10 minutes to separate the phases. Remove about 15 milliliters of the upper phase and proceed as directed in paragraph (e) of this section.

(e) *Procedure.* Fill the polarimeter tube with the blank solution prepared as described in paragraph (d) of this section. Place the tube in the polarimeter. Adjust the polarimeter to zero rotation using a light source with a wavelength of 365 nanometers. Use the same procedure to determine the optical rotation of both the sample solution and the working standard solution prepared as directed in paragraph (c) of this section.

(f) *Calculations.* Calculate the carbenicillin content (potency) of the sample on an anhydrous basis as follows:

$$\text{Micrograms of carbenicillin per milligram of sample} = \frac{\text{Degrees of rotation of sample solution} \times \text{weight of working standard} \times 100 \times \text{micrograms of carbenicillin in each milligram of the working standard}}{\text{Degrees of rotation of working standard solution} \times \text{weight of sample} \times (100-m)},$$

where: m = moisture content of the sample.

§ 436.301 Thin layer chromatography identity test for carbenicillin indanyl.

Using the sample solution prepared as described in the section for the antibiotic drug to be tested, proceed as described in paragraphs (a), (b), (c), and (d) of this section.

(a) *Equipment*—(1) *Chromatography tank.* A rectangular tank, approximately 9 x 9 x 3.5 inches lined with filter paper (3 millimeters in thickness).

(2) *Iodine vapor chamber.* A rectangular tank approximately 9 x 9 x 3.5 inches, with a suitable cover, containing iodine crystals.

(3) *Plates.* Use 20 x 20 centimeters thin layer chromatography plates coated with silica gel G or equivalent to a thickness of 250 microns.

(b) *Reagents*—(1) *Extraction solvent.* Mix ethyl acetate, acetone, pyridine, water, and acetic acid in volumetric proportions of 100:200:25:75:1.5 respectively.

(2) *Developing solvent.* Mix ethyl acetate, acetone, pyridine, water, and acetic acid in volumetric proportions of 300:400:25:75:2 respectively.

(3) *Ferric chloride-potassium ferricyanide reagent.* Immediately before use, mix 100 milliliters of a 1 percent ferric chloride solution in 1 percent hydrochloric acid with 100 milliliters of a 1 percent potassium ferricyanide solution and 75 milliliters of methanol.

(c) *Preparation of working standard solution.* Weigh an amount of the carbenicillin indanyl working standard equivalent to approximately 10 milligrams of carbenicillin into a 50-milliliter Erlenmeyer flask. Dissolve the material in sufficient extraction solvent to make a solution containing 1 milligram carbenicillin per milliliter.

(d) *Procedure.* Pour developing solvent into the bottom of the chromatography tank. Cover and seal the tank. Allow it to equilibrate for 1 hour. Prepare a plate as follows: On a line 2 centimeters from the base of the silica gel plate, and at intervals of 2 centimeters, spot 10 microliters of the standard solution and the sample solution. The plate should be air dried for 30 minutes. Place the plate into the chromatography tank. Allow the solvent front to travel about 15 centimeters from the starting line and then remove the plate from the tank. Heat the plate for 30 minutes at 80° C. in a circulating air oven and then allow the plate to cool to room temperature. Place the plate in the iodine vapor chamber for about 30 seconds, remove the plate and spray it with the ferric chloride-potassium ferricyanide reagent. Carbenicillin indanyl appears as a blue spot on a yellow-green background at an R_f of about 0.5. The test is satisfactory if the sample compares qualitatively with the standard.

§ 436.302 Clindamycin vapor phase chromatography.

(a) *Equipment.* Gas chromatograph equipped with a flame ionization detector: Barber-Colman 5,000 or equivalent.

(b) *Reagents.* (1) Pyridine, reagent grade, dried over sodium sulfate.

(2) Chloroform, reagent grade.

(3) Acetic anhydride, reagent grade, used as acteylating agent.

(4) Internal standard: Prepare a solution containing 3 milligrams of cholestane per milliliter in pyridine.

(c) *Typical conditions.* (1) Column: 4 feet x 4 millimeters ID, glass, with 1 percent SE–30 on Diatoport S (60/80 mesh), or equivalent.

(2) Temperatures: Column 200° C.; detector 215° C.; injection port, ambient temperature.

(3) Carrier gas: Helium approximately 120 milliliters per minute.

(4) Detector: Hydrogen flame—hydrogen at 120 pounds per square inch, air at 40 pounds per square inch.

(5) Sensitivity: 1,000; attenuation, 2 for clindamycin, 1 for internal standard; 2×10^{-4} amperes.

(d) *Preparation of clindamycin sample and working standard solutions.* Accurately weigh approximately 15 milligrams of sample or working standard into a glass-stoppered conical 15-milliliter centrifuge tube. Add 1.0 milliliter of chloroform, 1.0 milliliter of internal standard solution, and 0.6 milliliter of acetic anhydride. Agitate the tubes to insure dissolution of the sample and complete mixing of the liquids. Proceed as directed in paragraph (e) of this section.

(e) *Procedure.* Cover the top of each centrifuge tube with a plastic cap. Punch a small hole in the top of each cap to allow vapor to escape. Place the tubes in a 100° C. drying oven for 2.5 hours. Remove the tubes from the oven and allow to cool. Take the plastic cap from each tube and replace with the glass stopper. Centrifuge 10–15 minutes at 2,000–2,500 r.p.m. to separate the white solid from the liquid in the tube. Inject 0.5 microliter of the clear liquid into the gas chromatograph. Use the conditions and materials listed in paragraphs (a), (b), and (c) of this section. The conditions should be adequate to maintain a stable baseline and provide at least 60 percent deflection of the recorder scale by the clindamycin peak. The resolution of the peaks should be complete. The elution order is: Internal standard, clindamycin, and epiclindamycin (if present). Calculate the clindamycin content as directed in paragraph (f) of this section.

(f) *Calculations.* Calculate the clindamycin content of the sample as follows:

$$\text{Micrograms of clindamycin per milligram} = \frac{R_c \times W_s \times f}{R_s \times W_c},$$

where:

R_c = (Area of the clindamycin sample peak (at a retention time equal to that observed for the clindamycin standard)) / (Area of internal standard peak);

R_s = (Area of the clindamycin standard peak) / (Area of internal standard peak);

W_s = Weight of the clindamycin working standard in milligrams;

W_c = Weight of the sample in milligrams;

f = Potency of the clindamycin working standard in micrograms per milligram.

§ 436.303 Clindamycin content of clindamycin palmitate hydrochloride by vapor phase chromatography.

(a) *Equipment.* Gas chromatograph equipped with a flame ionization detector: Hewlett-Packard 7606 [4] or equivalent.

(b) *Reagents.* (1) Acetic anhydride, reagent grade.

(2) Pyridine, reagent grade.

(3) Chloroform, reagent grade.

(4) Internal standard: Prepare a solution containing 5 milligrams of cholesteryl benzoate per milliliter in chloroform.

[4] Available from: Hewlett Packard Co., P.O. Box 301, Loveland, CO 80537.

(c) *Typical conditions.* (1) Column: 6 feet x 2 millimeters ID, glass, with 1 percent UC–W98 on Chromosorb WHP (80/100 mesh) or equivalent.

(2) Temperatures: Column 275° C.; detector 290° C.; injection port 280° C.

(3) Carrier gas: Helium approximately 60 milliliters per minute.

(4) Detector: Hydrogen flame ionization—hydrogen at 12 pounds per square inch, air at 32 pounds per square inch.

(5) Sensitivity: 1,000; attenuation, 16; 1×10^{-9} amperes.

(d) *Preparation of clindamycin palmitate hydrochloride sample and working standard solutions.* Accurately weigh approximately 15 milligrams of both the sample and the working standard into separate glass-stoppered, conical 15-milliliter centrifuge tubes. Add 1.0 milliliter of internal standard solution, 1.0 milliliter of pyridine, and 0.5 milliliter of acetic anhydride to each tube. Agitate the tubes to insure dissolution and complete mixing of the liquids. Proceed as directed in paragraph (e) of this section.

(e) *Procedure.* Cover the top of each centrifuge tube with a plastic cap. Punch a small hole in the top of each cap to allow vapor to escape. Place the tubes in a 100° C. drying oven for 2.5 hours. Remove the tubes from the oven and allow to cool. Take the plastic cap from each tube and replace with the glass stopper. Centrifuge 10–15 minutes at 2,000–2,500 r.p.m. to separate the white solid from the liquid in the tube. Inject 1 microliter of the clear liquid into the gas chromatograph. Use the conditions and materials listed in paragraphs (a), (b), and (c) of this section. The conditions should be adequate to maintain a stable baseline and provide at least 40 percent deflection of the recorder scale by the clindamycin palmitate peak. The resolution of the peaks should be complete. The internal standard will be eluted before the clindamycin palmitate. Calculate the clindamycin content as directed in paragraph (f) of this section.

(f) *Calculations.* Calculate the clindamycin content of the sample as follows:

$$\text{Micrograms of clindamycin per milligram} = \frac{R_s \times W_s \times f}{R_r \times W_s},$$

where:

R_s = Area of the sample peak (at a retention time equal to that observed for the clindamycin palmitate hydrochloride standard) / Area of internal standard peak

R_r = Area of the clindamycin palmitate hydrochloride standard peak / Area of internal standard peak;

W_r = Weight of the clindamycin palmitate hydrochloride working standard in milligrams;

W_s = Weight of the sample in milligrams;

f = Micrograms of clindamycin activity per milligram of clindamycin palmitate hydrochloride working standard.

§ 436.304 Clindamycin phosphate vapor phase chromatography.

(a) *Equipment.* Gas chromatograph equipped with an electronic integrator and with a flame ionization detector that has a sensitivity of at least 1×10^{-10} amperes: Hewlett-Packard 7600[4] or equivalent.

(b) *Reagents.* (1) Trifluoroacetic anhydride.

(2) Intestinal alkaline phosphatase.

(3) pH 9.0 borate buffer: Transfer 3.1 grams of boric acid into a 1-liter volumetric flask containing 500 milliliters of water, mix, and add 21 milliliters of 1.0*N* sodium hydroxide and 10 milliliters of 0.1*M* magnesium chloride. Dilute to volume with water and mix well.

(4) Internal standard: Prepare a chloroform solution containing approximately 0.45 milligram hexacosane per milliliter.

(5) Anhydrous sodium carbonate.

(c) *Typical conditions.* (1) Column: 2 feet x 3 millimeters ID, glass, with 1 percent SE–30 on Diatoport S (80/100 mesh), or equivalent.

(2) Temperatures: Column, 180° C., detector, 215° C., injection port, ambient temperature.

(3) Carrier gas: Helium approximately 60 milliliters per minute.

(4) Detector: Hydrogen flame—hydrogen flow at 40 milliliters per minute. Air flow at 400 milliliters per minute.

(5) Sensitivity: 1×10^{-9} amperes.

(d) *Preparation of clindamycin phosphate sample solution.* Accurately weigh approximately 12 milligrams of the clindamycin phosphate sample into a 50-milliliter glass-stoppered centrifuge tube. Pipet 25 milliliters of the pH 9.0 borate buffer into the centrifuge tube. Add 10 milliliters chloroform and shake vigorously for 15 minutes. Centrifuge the resulting mixture and pipet a 20-milliliter aliquot of the aqueous phase into a 35-milliliter centrifuge tube. Add a weighed amount of intestinal alkaline phosphatase equivalent to 50 units of activity[5] and allow the solution to stand until the enzyme has completely dissolved. Place the tube into a water bath at 37° C.±2° C. for 2.5 hours. After the 2.5-hour hydrolysis, allow the solution to cool and proceed as directed in paragraph (f) of this section.

(e) *Preparation of the clindamycin hydrochloride standard solution.* Accurately weigh approximately 9 milligrams of the clindamycin hydrochloride working standard into a 35-milliliter glass-stoppered centrifuge tube and dissolve in 20 milliliters of pH 9.0 borate buffer. Proceed as directed in paragraph (f) of this section.

(f) *Procedure.* Add 10 milliliters of the internal standard solution to each sample and standard solution. Shake the centrifuge tubes vigorously for 30 minutes and centrifuge. Remove the aqueous layer and discard. Shake the tubes again; mix in an ultrasonic mixer for 2 minutes, then centrifuge. No emulsion should be present at this stage. Remove the remaining aqueous layer by suction and transfer a 3-milliliter aliquot of the chloroform layer to a 1-dram tablet vial containing approximately 1 gram of anhydrous sodium sulfate. Swirl the vial to dry the chloroform and transfer a 1-milliliter aliquot to another 1-dram tablet vial. Using a 0.25-milliliter pipet, add 0.25 milliliter of trifluoracetic anhydride to each of the vials and place into a water bath at 45° C.±2° C. for 30 minutes. Remove the vials from the bath, add about 10 granules of anhydrous sodium carbonate to each vial, and allow to stand for approximately 30 minutes. Centrifuge the vials for approximately 10 minutes at 5,000 r.p.m. Inject 2 microliters of each of the resulting solutions into the gas chromatograph. Use the conditions and materials listed in paragraphs (a), (b), and (c) of this section. The elution order is: Internal standard, clindamycin, and epiclindamycin (if present). Calculate the clindamycin content as directed in paragraph (g) of this section.

(g) *Calculations.* Calculate the clindamycin content of the sample as follows:

$$\text{Micrograms of clindamycin per milligram} = \frac{R_s \times W_s \times f}{R_r \times W_s},$$

where:

R_s = Area of the clindamycin sample peak (at a retention time equal to that observed for the clindamycin standard) / Area of internal standard peak

R_r = Area of the clindamycin standard peak / Area of internal standard peak;

W_r = Weight of the clindamycin working standard in milligrams;

W_s = Weight of the sample in milligrams;

f = Potency of the clindamycin working standard in micrograms per milligram.

§ 436.305 Thin layer chromatographic identity test for hetacillin.

(a) *Equipment*—(1) *Chromatography tank.* A rectangular tank, approximately 9 x 9 x 3.5 inches with a glass solvent trough on the bottom.

(2) *Plates.* Use 20 x 20 centimeter thin layer chromatography plates coated with Silica Gel G or equivalent to a thickness of 250 microns.

(b) *Developing solvent.* Mix 650 milliliters acetone with 100 milliliters distilled water, 100 milliliters benzene, and 25 milliliters acetic acid.

(c) *Spray solution.* Dissolve 300 milligrams of ninhydrin in 100 milliliters ethanol.

(d) *Preparation of spotting solutions*—(1) *Sample solution.* Use the sample solution prepared as described in the section for the particular product to be tested.

(2) *Reference solutions.* Prepare a solution containing 10 milligrams of an

[5] Defined such that 50 units hydrolyzes at least 20 micromoles of a clindamycin phosphate authentic sample under the assay conditions described in this section.

authentic hetacillin sample per milliliter in a 4:1 solution of acetone and 0.1*N* hydrochloric acid, and a solution of ampicillin standard at 1 mg/ml in the same solvent.

(e) *Procedure.* Spot a plate as follows: Apply approximately 10 microliters of the sample solution, 1 μl. of the reference hetacillin solution, and 1 μl. of the ampicillin reference solution on a line 1.5 centimeters from the base of the silica gel plate and at intervals of not less than 2.0 centimeters. Pour developing solvent into the glass through the bottom of the chromatography tank. After all spots are thoroughly dry, place the silica gel plate directly into the glass trough of the chromatography tank. Cover and seal the tank. Allow the solvent front to travel about 11.5 centimeters from the bottom of the plate, remove the plate from the tank, and allow to air dry. Apply the spray solution (do not saturate) and place immediately into an oven maintained at 90° C. Heat 15 minutes.

(f) *Evaluation.* Measure the distance the solvent front traveled from the starting line and the distance the spots are from the starting line. Calculate the R_f value by dividing the latter by the former. The sample and standard should have spots of corresponding R_f values.

§ 436.306 Lincomycin vapor phase chromatography.

(a) *Equipment.* Gas chromatograph equipped with a flame ionization detector; Barber-Colman 5000 or equivalent.

(b) *Reagents.* (1) Pyridine, reagent grade, kept over potassium hydroxide.

(2) Methanol, reagent grade, anhydrous.

(3) Ethanol, absolute, reagent grade.

(4) Internal standard: Prepare a solution containing 2 milligrams of tetraphenylcyclopentadienone per milliliter in pyridine.

(5) Silylating reagent: Mix (9+1) of hexamethyldisilazane and trimethylchlorosilane.

(c) *Typical conditions.* (1) Column: 4 feet x 3 millimeters ID, glass, with 3 percent SE-30 on Gas-Chrom Q (100/120 mesh), or equivalent.

(2) Temperatures: Column 225° C.; detector 280° C., injector 270° C.

(3) Carrier gas: Helium at 15 pounds per square inch.

(4) Detector: Hydrogen flame ionization—hydrogen at 20 pounds per square inch, air at 40 pounds per square inch.

(5) Sensitivity: 100; attenuation 2; current 2×10^{-9} amperes.

(d) *Preparation of lincomycin sample and working standard solutions.* Prepare the sample and working standard as follows: Weigh accurately an aliquot of about 40 milligrams into a 10-milliliter volumetric flask, add sufficient pyridine to dissolve, and make to mark. Transfer a 1-milliliter aliquot to a glass-stoppered conical centrifuge tube and proceed as directed in paragraph (e) of this section.

(e) *Procedure.* Add 0.2 milliliter of the silylating reagent to each centrifuge tube and allow to stand at least 30 minutes. Then add exactly 1 milliliter of the internal standard, shake well, and centrifuge. Inject 5 microliters of the supernatant into the gas chromatograph. Use the typical conditions and materials listed in paragraphs (a), (b), and (c) of this section. The conditions should be adequate to provide at least 60 percent scale deflection with the lincomycin peak and to maintain a stable base line. The resolution of the peaks should be complete. The elution order is lincomycin B, lincomycin, and the internal standard. If necessary, adjust the current setting for the lincomycin B peak to give a satisfactory response relative to that of the lincomycin peak. Calculate the lincomycin content and lincomycin B content as directed in paragraph (f) of this section.

(f) *Calculations.*

$$\text{Lincomycin content of sample in micrograms per milligram} = \frac{(R_u)(W_s)(f)}{(R_s)(W_u)}.$$

Where:

R_u = Area of the lincomycin sample peak / Area of internal standard peak;

R_s = Area of the lincomycin standard peak / Area of internal standard peak;

W_s = Weight of the lincomycin working standard in milligrams;

W_u = Weight of the sample in milligrams;

f = Potency of lincomycin working standard in micrograms per milligram.

$$\text{Percent lincomycin B content} = \frac{B}{A+B} \times 100,$$

where:

A = Area of lincomycin peak of the sample;

B = Area of lincomycin B peak of the sample corrected for the attenuation adjustment.

§ 436.307 Spectinomycin vapor phase chromatography.

(a) *Equipment.* Gas chromatograph equipped with a flame ionization detector; Barber-Coleman 5,000 or equivalent.

(b) *Reagents.* (1) Dimethylformamide, reagent grade, kept dry over anhydrous sodium sulfate.

(2) Internal standard: Prepare a solution containing 2 milligrams of triphenylantimony per milliliter in dry dimethylformamide.

(3) Silylating reagent: Hexamethyldisilazane.

(c) *Typical conditions.* (1) Column: 4 feet by 4 millimeters ID, glass, with 5 percent SE-52 on Diatoport S (80/100 mesh), or equivalent.

(2) Temperatures: Column 215° C.; detector 270° C.; injection port 265° C.

(3) Carrier gas: Helium 93 milliliters per minute at 15 pounds per square inch.

(4) Detector: Hydrogen flame—hydrogen at 20 pounds per square inch, air at 40 pounds per square inch.

(5) Sensitivity: 1,000; attenuation, 10 for both spectinomycin and internal standard; 2×10^{-9} amperes.

(d) *Preparation of spectinomycin sample and working standard—*(1) *Working standard and bulk antibiotic solutions.* (i) Accurately weigh approximately 30 milligrams of sample or working standard into separate glass-stoppered 25-milliliter Erlenmeyer flasks.

(ii) Add 10 milliliters of the internal standard solution and 1.0 milliliter of hexamethyldisilazane to each flask. Agitate the flasks to insure dissolution of the sample and working standard and complete mixing of the liquids. Shake the flasks intermittently for 1 hour. Proceed as directed in paragraph (e) of this section.

(2) *Finished product solutions.* Prepare the sample for assay as directed in the individual section for each antibiotic product to be tested.

(e) *Procedure.* Inject 2.5 microliters of each solution into the gas chromatograph. Use the conditions and materials listed in paragraphs (a), (b), and (c) of this section. The conditions should be adequate to maintain a stable base line and provide at least 60 percent deflection of the recorder scale by the spectinomycin peak. The resolution of the peaks should be complete. The internal standard will be eluted before spectinomycin. Calculate the spectinomycin content as directed in paragraph (f) of this section.

(f) *Calculations.* Calculate the spectinomycin content of the sample as follows:

$$\text{Micrograms of spectinomycin per milligrams} = \frac{R_u \times W_s \times f}{R_s \times W_u}$$

where:

R_u = Area of spectinomycin sample peak (at a retention time equal to that observed for the spectinomycin standard) / Area of internal standard peak

R_s = Area of the spectinomycin standard peak / Area of internal standard peak

W_s = Weight of the spectinomycin working standard in milligrams;

W_u = Weight of the sample in milligrams;

f = Potency of the spectinomycin working standard in micrograms per milligram.

§ 436.308 Paper chromatography identity test for tetracyclines.

(a) *Equipment*—(1) *Sheet (chromatographic)*. Whatman No. 1 filter paper for chromatography, 20 x 20 centimeters.

(2) *Chamber (chromatographic)*. Cylindrical glass chromatographic jar, 25 x 12 x 25 centimeters, with a ground-glass lid.

(3) *Preparation of solutions*—(i) *pH 3.5 buffer*. Mix 13.93 volumes of 0.1*M* citric acid with 6.07 volumes of 0.2*M* of disodium phosphate.

(ii) *Solvent (organic phase)*. Mix chloroform, nitromethane, and pyridine in volumetric proportions of 10:20:3, respectively.

(b) *Procedure*. Fill the chamber to a depth of 0.6 centimeter with freshly prepared solvent. Draw a starting line about 2.5 centimeters from and parallel to the bottom of the sheet. Wet the sheet thoroughly with the pH 3.5 buffer and blot it firmly between sheets of absorbent paper. Starting about 5 centimeters from the edge of the sheet and at 1.5-centimeter intervals, apply to the starting line 2 microliters each of standard solution (1 milligram per milliliter), sample solution (1 milligram per milliliter), and a 1:1 mixture of the standard and sample solutions. Allow a few minutes for the sheet to dry partially, and while still damp place it in the chamber with the bottom edge touching the solvent. When the solvent front has risen about 10 centimeters, remove the sheet from the chamber. Examine the dried sheet under a strong source of ultraviolet light and record the position of any fluorescent spots. Measure the distance the solvent front traveled from the starting line and the distance that the fluorescent spots are from the starting line. Calculate the R value by dividing the latter by the former.

§ 436.309 Anhydrotetracyclines and 4-epianhydrotetracycline.

Determination of 4-epianhydrotetracycline and anhydrotetracyclines in tetracycline, tetracycline hydrochloride, tetracycline phosphate, and in dosage forms thereof is as follows:

(a) *Screening procedure for total anhydrotetracyclines content*—(1) *Sample solution preparation*—(i) *Bulk packaged for repacking or for use in the manufacture of another drug*. Accurately weigh approximately 50 milligrams of the sample into a 50-milliliter volumetric flask and add 10 milliliters of 0.1*N* hydrochloric acid. Shake until sample is completely dissolved, and then dilute to volume with water.

(ii) *Sterile dispensing containers*. Proceed as directed in paragraph (a) (1) (i) of this section.

(iii) *Capsules*. Transfer a representative quantity of capsule contents equivalent to 250 milligrams of tetracycline hydrochloride to a 250-milliliter volumetric flask. Add 50 milliliters of 0.1*N* hydrochloric acid and shake on a mechanical shaker for 5 minutes. Dilute to volume with water and filter through a fluted filter paper. Discard the first 20 milliliters of filtrate and collect the next 20 milliliters.

(iv) *Tablets*. Grind a representative number of tablets to a fine powder. Transfer an amount of the powder equivalent to 250 milligrams of tetracycline hydrochloride to a 250-milliliter volumetric flask. Add 50 milliliters of 0.1*N* hydrochloric acid and shake on a mechanical shaker for 5 minutes. Dilute to volume with water and filter through a fluted filter paper. Discard the first 20 milliliters of filtrate and collect the next 20 milliliters.

(v) *Oral powders and suspensions*. Proceed as described in paragraph (b) of this section.

(2) *Test procedure*. Using a suitable spectrophotometer, determine the absorbance of the sample solution prepared as directed in paragraph (a) (1) of this section at 430 millimicrons using 0.02*N* hydrochloric acid as a blank. Then accurately dilute 1.0 milliliter of the sample solution to 100 milliliters with 0.02*N* hydrochloric acid and determine the absorbance of this solution at 356 millimicrons, using 0.02*N* hydrochloric acid as a blank.

(3) *Calculations*.

$$\text{Percent anhydrotetracyclines} = \frac{[a430 - (a356 \times 0.0019)] \times 100}{180}$$

where:

$a430$ = Absorptivity (1%, 1 cm.) of sample at 430 millimicrons;

$$\text{For bulk, absorptivity} = \frac{\text{Absorbance} \times 50 \times 10;}{\text{Milligrams of sample}}$$

For sterile dispensing containers, capsules, and tablets; absorptivity = Absorbance × 10;

$a356$ = Absorptivity (1%, 1 cm.) of sample at 356 millimicrons;

$$\text{For bulk, absorptivity} = \frac{\text{Absorbance} \times 50 \times 1000;}{\text{Milligrams of sample}}$$

For sterile dispensing containers, capsules and tablets; absorptivity = Absorbance × 1,000;

0.0019 = Absorbance ratio ($A430/A356$) observed with tetracycline;

180 = Absorptivity (1%, 1 cm.) of anhydrotetracycline hydrochloride at 430 millimicrons.

(4) *Evaluation*. If the total anhydrotetracyclines content determined by the screening procedure described in paragraph (a) of this section exceeds 2 percent for bulks and 3 percent for injectables, tablets, and capsules, perform the determination for anhydrotetracyclines and 4-epianhydrotetracycline described in paragraph (b) of this section. If the results of the test described in paragraph (a) of this section for total anhydrotetrayclines content are within the required limits in the case of bulks, injectables, tablets, and capsules, these results may be submitted in lieu of the results of the test for 4-epianhydrotetracycline and that test as described in paragraph (b) of this section need not be performed.

(b) *Determination of anhydrotetracyclines content and 4-epianhydrotetracycline content*—(1) *Apparatus and reagents*—(i) Chromatographic tubes (15 millimeters ID x 170 millimeters long having an outlet tube 4 millimeters ID x 50 millimeters long).

(ii) pH meter standardized at pH 7.0 and at pH 10.0.

(iii) Diatomaceous earth, acid-washed (Celite 545 or equivalent).

(iv) EDTA buffer: Dissolve 0.1 mole ethylenediaminetetraacetic acid disodium salt in 800 milliliters of water. Adjust to pH 7.8 with ammonium hydroxide, reagent grade, and dilute to 1 liter with water.

(v) Chloroform, spectrophotometric grade.

(vi) Diluted ammonium hydroxide: Mix 1 volume of ammonium hydroxide, reagent grade, with 9 volumes of distilled water.

(vii) 0.1*N* hydrochloric acid.

(viii) 1.0*N* hydrochloric acid.

(2) *Preparation of support phase*. Add 5 milliliters of EDTA buffer to 10 grams of diatomaceous earth and mix until the diatomaceous earth is uniformly moistened. It will no longer be free-flowing.

(3) *Preparation of sample solutions*. Prepare the sample solutions as follows:

(i) *Tetracycline, tetracycline phosphate complex, and tetracycline hydrochloride bulk packaged for repacking or for use in the manufacture of another drug*. Place an amount of sample equivalent to 250 milligrams of tetracycline hydrochloride into a 50-milliliter beaker and dissolve in 10 milliliters of 0.1*N* hydrochloric acid. Immediately adjust the pH to 7.8 with the diluted ammonium hydroxide, and if necessary, with 1.0*N* hydrochloric acid and 0.1*N* hydrochloric acid. Quantitatively transfer this solution to a 50-milliliter volumetric flask by rinsing the beaker with EDTA buffer, fill to volume with EDTA buffer and shake well. Use this solution without delay to prepare a column as directed in paragraph (b) (4) of this section.

(ii) *Capsules*. Proceed as directed in paragraph (b) (3) (i) of this section, except pool the contents of a representative number of capsules and use an amount of the pooled capsule contents equivalent to 250 milligrams of tetracycline hydrochloride.

(iii) *Tablets*. Proceed as directed in paragraph (b) (3) (i) of this section, except grind tablets to a powder in a small mortar and use an amount of powder equivalent to 250 milligrams of tetracycline hydrochloride.

(iv) *Oral suspension and pediatric drops*. Place 5 milliliters of oral suspension equivalent to 125 milligrams of tetracycline hydrochloride or 2 milliliters of pediatric drops equivalent to 200 milligrams of tetracycline hydrochloride into a 50-milliliter beaker and add sufficient 0.1*N* hydrochloric acid to make 10 milliliters. Quickly adjust the pH to 7.8 with the diluted ammonium hydroxide, and if

necessary, with 1*N* hydrochloric acid and 0.1*N* hydrochloric acid. Quantitatively transfer this solution to a 25-milliliter flask by rinsing the beaker with EDTA buffer, fill to volume with EDTA buffer, and shake well. Use this solution without delay to prepare a column as directed in paragraph (b)(4) of this section.

(v) *Oral powders.* Reconstitute as directed in the labeling and proceed as directed in paragraph (b)(3)(iv) of this section.

(vi) *Sterile dispensing containers.* Proceed as directed in paragraph (b)(3)(i) of this section.

(4) *Column preparation.* Pack support phase into the chromatographic tube by increments and firmly tamp down each increment. Do not use any glass wool in the column outlet. Add enough support phase to the column to reach a height of 9 to 11 centimeters; then add 1 milliliter of sample solution to 1 gram of diatomaceous earth in a small beaker, and mix thoroughly. Pack the sample: diatomaceous earth mixture on top of the column. Dry wash the beaker with support phase and pack an additional 1-centimeter layer of support phase on top of the sample layer.

(5) *Column elution and fraction collection.* Within 30 minutes after preparing the column, elute with chloroform. Collect 5 successive fractions of 5 milliliters, 5 milliliters, 10 milliliters, 10 milliliters, and 5 milliliters. During elution, two clear separate yellow bands will appear on the column. The first band is anhydrotetracyclines and will almost always elute in the first 5-milliliter fraction, but occasionally in the first and second 5-milliliter fractions. The second band is 4-epianhydrotetracycline and will elute in the remaining fractions. Label the fraction or fractions containing the first yellow band anhydrotetracyclines. Label the fractions after the first yellow band 4-epianhydrotetracycline. Determine the absorbance of each fraction at a wavelength of 438 nanometers using a suitable spectrophotometer equipped with a 1.0-centimeter cell and chloroform as the blank. If necessary, make appropriate dilutions with choloroform to obtain a readable value.

(6) *Calculations*—(i) *Percent anhydrotetracyclines.* Calculate the percent anhydrotetracyclines as follows:

$$\text{Number of milligrams of anhydrotetracyclines in each fraction containing anhydrotetracyclines} = \frac{A \times b \times c}{20.28}$$

where:

A = Absorbance of the sample solution at 438 nanometers;
b = Volume of fraction in milliliters;
c = Dilution factor of the fraction (for example, if 2 milliliters of the fraction are diluted to 10 milliliters for reading, c will be 5).
20.28 = Absorptivity (1 milligram per milliliter, 1 centimeter) of anhydrotetracyclines in chloroform at 438 nanometers.

$$\text{Total weight of anhydrotetracyclines in the sample} = \text{Sum of weights of anhydrotetracyclines in the fractions labeled anhydrotetracyclines} \times \text{Number of milliliters in the sample solution}$$

$$\text{Percent anhydrotetracyclines in tetracycline, tetracycline hydrochloride, tetracycline phosphate complex bulk packaged for repacking or for use in the manufacture of another drug} = 100 \times \frac{\text{Total weight of anhydrotetracyclines in the sample}}{\text{Weight of the sample}}$$

$$\text{Percent anhydrotetracyclines in dosage forms} = 100 \times \frac{\text{Total weight of anhydrotetracyclines in the sample}}{\text{Labeled tetracycline content of the sample}}$$

(ii) *Percent 4-epianhydrotetracycline.* Calculate the percent 4-epianhydrotetracycline as follows:

$$\text{Number of milligrams of 4-epianhydrotetracycline in each fraction labeled 4-epianhydrotetracycline} = \frac{A \times b \times c}{20.08}$$

where:

A = Absorbance of the sample solution at 438 nanometers;
b = Volume of the fraction in milliliters;
c = Dilution factor of the fraction (for example, if 2 milliliters of the fraction are diluted to 10 milliliters for reading, c will be 5);
20.08 = Absorptivity (1 milligram per milliliter, 1 centimeter) of 4-epianhydrotetracycline in chloroform at 438 nanometers.

$$\text{Total weight of 4-epianhydrotetracycline in the sample} = \text{Sum of weights of 4-epianhydrotetracycline in the fractions labeled 4-epianhydrotetracycline} \times \text{Number of milliliters in the sample solution}$$

$$\text{Percent 4-epianhydrotetracycline in tetracycline, tetracycline hydrochloride, tetracycline phosphate complex bulk packaged for repacking or for use in the manufacture of another drug} = 100 \times \frac{\text{Total weight of 4-epianhydrotetracycline in the sample}}{\text{Weight of the sample}}$$

$$\text{Percent 4-epianhydrotetracycline in dosage forms} = 100 \times \frac{\text{Total weight of 4-epianhydrotetracycline in the sample}}{\text{Labeled tetracycline content of the sample}}$$

(7) *Limits for 4-epianhydrotetracycline.* The 4-epianhydrotetracycline content of tetracycline, tetracycline hydrochloride, and tetracycline phosphate complex bulk drugs and all oral and injectable dosage forms containing these tetracyclines shall not exceed 2 percent for bulk drugs, 3 percent for injectables, tablets, capsules and oral powders and 5 percent for oral suspension.

§ 436.310 Thin layer chromatography identity test for mitomycin.

(a) *Equipment*—(1) *Chromatography tank.* A rectangular tank, approximately 9 x 9 x 3.5 inches, lined with filter paper and with a solvent trough on the bottom.

(2) *Plates.* Use 20 by 20 centimeter thin layer chromatography plates coated with Silica Gel G or equivalent, to a thickness of 250 microns.

(b) *Reagents*—(1) *Developing solvent.* Mix *n*-butanol, glacial acetic acid, and water in volumetric proportions of 4:2:1, respectively.

(2) *Spray solution.* Prepare a one-percent solution of ninhydrin in ethanol.

(c) *Preparation of spotting solutions.* Prepare solutions of the sample and working standard, each containing 1 milligram of mitomycin per milliliter, in water.

(d) *Procedure.* Pour developing solvent into the bottom of the chromatography tank. Cover and seal the tank. Allow it to equilibrate for 30 minutes. Prepare a plate as follows: Apply spotting solutions on a line 2.5 centimeters from the base of the silica gel plate and at points 2.0 centimeters apart. Apply approximately 2 microliters of the working standard solution to points 1 and 3. When these spots are dry, apply approximately 2 microliters of sample solution to points 2 and 3. After all spots are thoroughly dry, place the silica gel plate into the trough in the chromatography tank. Cover and seal the tank tightly. Allow the solvent front to travel about 10 centimeters from the starting line. Remove the plate and allow it to air dry. After the plate is dry, spray lightly with the spray solution. Heat the plate in an oven at 110° C. for 10–15 minutes. Mitomycin appears as a pink spot.

(e) *Evaluation.* The sample and standard should have spots of corresponding R_f value (approximately 0.51), and standard and sample combined should appear as a single spot of corresponding R_f value.

Subpart G—Chemical Tests for Nonantibiotic Active Ingredients

§ 436.400 Thin layer chromatographic identity test for iodochlorhydroxyquin.

(a) *Equipment*—(1) *Chromatography tank.* A rectangular tank, approximately 9 x 9 x 3.5 inches with a glass solvent trough on the bottom.

(2) *Plates.* Use 20 x 20 centimeter thin layer chromatography plates coated with Silica Gel G or equivalent to a thickness of 250 microns.

(b) *Developing solvent.* Mix benzene and methanol in volumetric proportions of 90:10.

(c) *Preparation of spotting solutions*—(1) *Sample solution.* Use the sample solution prepared as described in the section for the particular product to be tested.

(2) *Reference solution.* Prepare a solution containing 0.5 milligram of iodochlorhydroxyquin U.S.P. reference standard per milliliter in acetone.

(d) *Procedure.* Pour developing solvent into the glass trough on the bottom of the chromatography tank. Cover and seal the tank. Allow it to equilibrate for 1 hour. Spot a plate as follows: Apply approximately 10 microliters each of the sample solution and of the reference solution on a line 2.0 centimeters from the base of the silica gel plate and at intervals of not less than 2.0 centimeters. After all spots are thoroughly dry, place the silica gel plate directly into the glass trough of the chromatography tank. Cover and reseal the tank. Allow the solvent front to travel about 15 centimeters from the starting line, remove the plate from the tank, and allow to air dry. Examine under a strong source of ultraviolet light. The sample and standard are visible as dark spots.

(e) *Evaluation.* Measure the distance the solvent front traveled from the starting line and the distance the spots are from the starting line. Calculate the R_f value by dividing the latter by the former. The sample and standard should have spots of corresponding R_f values (0.55 to 0.60).

Subpart H—Tests for Specific Antibiotic Dosage Forms

§ 436.500 Penicillin in oil and wax.

(a) *Potency.* Proceed as directed in § 440.80a(b)(1) of this chapter except paragraph (b)(1)(ix) thereof and, in lieu of the directions in § 440.80a(b)(1)(iv) of this chapter prepare sample as follows: Liquefy the sample by warming, thoroughly mix, and withdraw 1.0 milliliter using a sterile syringe equipped with an 18-gauge needle. Transfer to a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the separatory funnel vigorously to bring about complete mixing of the material with the ether. Shake with a 25-milliliter portion of 1 percent phosphate buffer at pH 6.0. Remove the buffer layer and repeat the extraction with three 25-milliliter quantities of buffer. Combine the extracts and make the proper estimated dilutions in 1 percent phosphate buffer at pH 6.0. The sample may also be prepared by transferring aseptically 1.0 milliliter of the penicillin in oil and wax to a blending jar containing 100 milliliters of 1 percent phosphate buffer at pH 6.0. Using a high-speed blender, blend this mixture for 1 minute and then make the proper estimated dilutions in 1 percent phosphate buffer at pH 6.0. If the label represents the potency of the penicillin in oil and wax as 200,000 units per milliliter or less, it is satisfactory if it is 85 percent or more of the potency so represented; if represented as more than 200,000 units per milliliter, it is satisfactory if it is 90 percent or more of the potency so represented.

(b) *Sterility.* Proceed as directed in § 436.20, using the method described in paragraph (e)(2) of that section, except using medium B in lieu of medium A.

(c) *Moisture*—(1) *Reagents*—(i) *Karl Fischer reagent.* Preserve the reagent in glass-stoppered bottles and use from an all glass automatic burette, protecting the solution from the moisture in the air.

(ii) *Water-methanol solution.* Use methanol containing approximately 1 mg. of water per milliliter. Store the solution in a glass bottle attached to an automatic burette and protect from moisture in the air at all times.

(2) *Standardization of Karl Fischer reagent.* Add a known volume of the Karl Fischer reagent to a suitable titrating vessel which has been previously dried at 105° C. and cooled in a desiccator. Introduce a mechanical stirrer and two platinum electrodes which are connected to a suitable electrometric apparatus for measurement of the endpoint. Start the stirrer and titrate with the water-methanol solution until the endpoint is reached. Calculate the milliliters of Karl Fischer reagent equivalent to each milliliter of water-methanol.

Add an accurately weighed quantity of water (approximately 50 milligrams) to a dry titrating vessel, add an excess of the Karl Fischer reagent and back titrate with the water-methanol solution as above. Calculate the milligrams of water equivalent to each milliliter of the Karl Fischer reagent. Standardize the Karl Fischer reagent in this manner daily.

$$e=\frac{w}{v_1-v_2 f}$$

where:

e=milligrams of water equivalent to 1 ml. Karl Fischer reagent.
w=weight of water in milligrams.
v_1=volume of Karl Fischer reagent used.
v_2=volume of methanol used.
f=volume ratio of Karl Fischer reagent to water-methanol solution.

(3) *Procedure.* Transfer 1.0 milliliter of the penicillin in oil and wax to a dry titrating vessel, add 10 milliliters of dry chloroform and an excess of the Karl Fischer reagent and back titrate with the water-methanol solution until the endpoint is reached. Transfer 10 milliliters of the dry chloroform used to a dry titrating vessel, add an excess of Karl Fischer reagent, and titrate with the water-methanol as above. Calculate the milliliters of Karl Fischer reagent equivalent to 10 milliliters of chloroform.

$$\text{Percent moisture}=\frac{(v_1-v_2 f-b)\times e\times 100}{s\times 1000}$$

where:

b=milliliters Karl Fischer reagent equivalent to 10 ml. of chloroform.
s=volume of the sample in milliliters.

(d) *Measurement of penicillin particle size.* Vigorously shake the container to obtain an even suspension of the penicillin particles and immediately withdraw therefrom approximately 0.5 milliliter of the drug into a clean, dry, tuberculin syringe using a dry 18-gauge needle. Discard approximately the first 5 drops of the mixture extruded from the needle and then extrude approximately 1 minim of the remaining mixture into a test tube containing 3 to 4 milliliters of light mineral oil. Thoroughly mix the contents of the tube and by means of a bacteriological loop (2 millimeters inside diameter, 22 gauge wire), immediately place one loopful of the suspension on each ruled chamber of a bright line hemocytometer. (It is not necessary to use a cover slip.) Confirm by means of the low power objective of the microscope the even distribution of particles over the ruled areas of both chambers and repeat with another loopful of the suspension if even dispersion is not obtained. Use a magnification of 430 or 440 diameters and a calibrated ocular micrometer to measure the penicillin particles. For the purpose of measurement and calculation, the predominant type of crystals observed shall be considered to represent the type of crystals present and the thickness and density of all particles shall be considered constant. Center a large penicillin particle in the microscopic field; measure the particle and all other particles in the field and repeat this operation on other fields until at least 200 particles are measured. Particles of less than 5 microns in length are disregarded. The grouping of the particles by length, the midpoint, the ratio of the midpoints, and the square of the ratio of the midpoints for each group are tabulated below:

Group	Length in microns	Midpoint	Ratio of midpoints	(Ratio)²
1	5-14	9.5	1.00	1.00
2	15-29	22.0	2.31	5.34
3	30-49	39.5	4.16	17.31
4	50-69	59.5	6.26	39.19
5	70-99	84.5	8.89	79.03
6	100-149	124.5	13.10	171.61
7	150-199	174.5	18.36	337.09
8	200-249	224.5	23.63	558.38
9	250-300	275.0	28.95	838.10

Calculate the percent particles in each group from the total number measured. Determine the percent relative weight for each group as follows:

Plate type particles. Relative weight=(ratio)²×% of total particles in group.

$$\%\text{ relative weight}=\frac{\text{relative weight}\times 100}{\text{total relative weight}}$$

Rod-shaped particles. In the case of rod-shaped particles measure the width as well as the length.

Relative weight=ratio × average width × % of total particles in group

$$\%\text{ relative weight}=\frac{\text{relative weight}\times 100}{\text{total relative weight}}$$

When examined by the method described in this section not less than 50 percent of the total relative weight of the penicillin in the drug consists of penicillin having a particle size of not less than 50 microns in length.

§ 436.503 Procaine penicillin and buffered crystalline penicillin for aqueous injection.

(a) *Total potency (except in single-dose container), sterility, moisture, pyro-*

gens, toxicity, pH. Proceed as directed in § 440.274b(b) of this chapter.

(b) *Buffered crystalline penicillin content*—(1) *Preparation of the solution for assay.* Add the indicated amount of distilled water to the contents of a vial of the sample, and shake well. Withdraw one dose of the suspension with a hypodermic syringe and place in a 10-milliliter volumetric flask. Add 20-percent sodium sulfate solution almost to the mark, centrifuge sufficiently to see the meniscus, make to volume with 20-percent sodium sulfate solution, shake well, and centrifuge to obtain a clear or reasonably clear solution. Dilute a 5.0-milliliter aliquot of this clear solution with 1-percent phosphate buffer, pH 6.0, to give a solution for assay of approximately 2,000 units per milliliter.

(2) *Iodometric assay for total penicillin in the solution for assay.* Determine the quantity of penicillin in the solution for assay by the iodometric assay procedure described in § 440.80a(b)(5)(iv)(*a*) of this chapter.

(3) *Colorimetric determination of procaine penicillin in the solution for assay.* Transfer an aliquot of the solution for assay to a 50-milliliter volumetric flask. Determine the quantity of procaine penicillin in this solution by the following method:

(i) *Reagents*—(*a*) *Sodium nitrite solution.* Dissolve 0.1 gram of sodium nitrite in 100 milliliters of distilled water. Prepare fresh solution every week and store under refrigeration.

(*b*) *Ammonium sulfamate solution.* Dissolve 0.5 gram of ammonium sulfamate in 100 milliliters of distilled water and store under refrigeration.

(*c*) *N-(1-naphthyl)-ethylenediamine solution.* Dissolve 0.1 gram of *N*-(1-naphthyl)-ethylenediamine dihydrochloride in 100 milliliters of distilled water. Prepare fresh solutions every week and store under refrigeration.

(*d*) *Standard procaine solution.* Prepare a standard solution containing 27.55 milligrams of procaine hydrochloride U.S.P. in a liter of distilled water (each milliliter of the standard solution is equivalent to 60 units of procaine penicillin).

(ii) *Standards.* Transfer, respectively, 1.0, 2.0, 3.0, 4.0, and 5.0 milliliters of the standard solution and 5.0 milliliters of distilled water to each of six 50-milliliter volumetric flasks. Add 4.0, 3.0, 2.0, and 1.0 milliliters of water to the first four flasks, respectively, to give each a volume of 5.0 milliliters.

(iii) *Procedure.* To each flask of the standards and the solution for assay add 0.5 milliliter of 4 *N* HCl, 1.0 milliliter of the sodium nitrite solution, 1.0 milliliter of the ammonium sulfamate, and 1.0 milliliter of the *N*-(1-naphthyl)-ethylenediamine solution. Mix and wait two minutes after each addition. Make each flask to volume of 50 milliliters with distilled water. Determine the absorbency of the colored solutions at 550 mμ in a suitable photo electric colorimeter. The instrument is balanced so that the zero concentration reads zero absorbency. Plot the standard curve on coordinate graph paper. Obtain the procaine penicillin content of the solution for assay directly from the point on the standard curve corresponding to its absorbency.

(4) The content of buffered crystalline penicillin in one dose of the product is calculated as follows:

$$A=(B-C)F,$$

where:

A = buffered crystalline penicillin content of the product.
B = total number of units of penicillin per milliliter as determined in paragraph (b)(2) of this section.
C = number of units of procaine penicillin per milliliter as determined in paragraph (b)(3) of this section.
F = appropriate dilution factor depending on the dilution made in the preparation of the solution for assay.

The content of buffered crystalline penicillin in the batch is satisfactory when determined by the method described in this paragraph if it is not less than 85 percent of that which it is represented to contain.

(c) *Procaine penicillin.* The procaine penicillin content of the batch is the difference between the total potency determined by the method described in paragraph (a) or (d) of this section and the content of the buffered crystalline penicillin determined by the method described in paragraph (b) of this section. The procaine penicillin content of the batch is satisfactory when determined by the method described in this paragraph if it is not less than 85 percent of that which it is represented to contain.

(d) *Total potency of a one-dose container.* Wash out the material remaining in the 10-milliliter volumetric flask referred to in paragraph (b)(1) of this section with 1-percent phosphate buffer, pH 6.0. Dilute to give a concentration of approximately 2,000 units per milliliter, and assay by the iodometric method described in § 440.80a(b)(5)(i)(*a*) of this chapter. Obtain the total potency by adding the number of units found in this solution (units per milliliter × volume) to the number of units found (units per milliliter × volume) in the solution assayed in accordance with paragraph (b)(2) of this section.

§ 436.504 Penicillin-bacitracin ointment.

(a) *Potency*—(1) *Penicillin content.* Proceed as directed in § 141a.8(a) of this chapter, except the last sentence of that paragraph. Its content of penicillin is satisfactory if it contains not less than 85 percent of the number of units it is represented to contain.

(2) *Bacitracin content.* Proceed as directed in § 448.510a(b)(1) of this chapter, except that sufficient penicillinase is added to the sample under test to completely inactivate the penicillin present. Its content of bacitracin is satisfactory if it contains not less than 85 percent of the number of units it is represented to contain.

(b) *Moisture.* Proceed as directed in § 436.201.

§ 436.505 Penicillin-streptomycin-bacitracin ointment; penicillin-dihydrostreptomycin-bacitracin ointment; penicillin-streptomycin-bacitracin methylene disalicylate ointment; penicillin-dihydrostreptomycin-bacitracin methylene disalicylate ointment.

(a) *Potency*—(1) *Content of penicillin, streptomycin, and dihydrostreptomycin.* Proceed as directed in § 141a.35(a) of this chapter.

(2) *Bacitracin content.* Proceed as directed in § 448.510a(b)(1) of this chapter, except that:

(i) Sufficient penicillinase is added to the sample under test to completely inactivate the penicillin present.

(ii) Use as the test organism the streptomycin dihydrostreptomycin resistant strain of either Micrococcus flavus (ATCC 10240A)[1] or Sarcina subflava (ATCC 7468/d),[1] grown and maintained in media containing 500 micrograms of streptomycin or dihydrostreptomycin per milliliter of media, or calculate from the quantity of streptomycin or dihydrostreptomycin found, using the method prescribed by paragraph (a)(1) of this section, the quantity that would be present when the sample is diluted to contain one unit of bacitracin (labeled potency) per milliliter. Prepare the bacitracin standard curve by adding the calculated quantity of streptomycin or dihydrostreptomycin to each concentration of bacitracin used for the curve. Use this standard curve to calculate the bacitracin content of the sample.

(3) *Bacitracin methylene disalicylate content.* Proceed as directed in paragraph (a)(2) of this section, except prepare the sample as follows: Place a representative portion of the sample (usually approximately 1 gram, accurately weighed) or the entire contents of a single-dose container in blending jar, add 99 milliliters of a 2.0-percent aqueous solution of sodium bicarbonate and 1 milliliter of a 10-percent aqueous solution of polysorbate 80 and blend for 3 minutes in a high-speed blender. Allow the foam to subside, remove an aliquot of the solution, and dilute to 1 unit per milliliter with 1.0-percent phosphate buffer, pH 6.0.

(b) *Moisture.* Proceed as directed in § 436.201.

436.506 Benzathine penicillin G and buffered crystalline penicillin for aqueous injection.

(a) *Total potency (except in single-dose containers).* Proceed as directed in § 440.80a(b)(1) of this chapter, except if the bioassay method is used prepare the sample by diluting 1.0 milliliter of the drug suspension with sufficient dimethyl formamide, formamide, or methyl alcohol to dissolve the benzathine penicillin. Make to 100 milliliters with buffer. Shake well and dilute to 1.0 unit per milliliter.

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

If the iodometric method is used, proceed as directed in § 440.55a(b) of this chapter, except in preparing the blank solution dilute 1.0 milliliter of the drug suspension to 250 milliliters with 1-percent phosphate buffer at pH 6.0. In preparing the solution for inactivation dissolve 1.0 milliliter of the drug suspension in approximately 20 milliliters of 0.5 *N* NaOH. Allow to stand for 15 minutes. Dilute to 250 milliliters with distilled water. Pipette a 2.0-milliliter aliquot into a 125-milliliter glass-stoppered Erlenmeyer flask and add 2.0 milliliters 1.2 *N* HCl and 10 milliliters 0.01 *N* iodine.

(b) *Buffered crystalline penicillin content.* Place 1.0 milliliter of the drug suspension in a 10-milliliter volumetric flask and add 20 percent sodium sulfate to make 10 milliliters. Shake well and centrifuge to obtain a clear, or reasonably clear, solution. Dilute a 5.0-milliliter aliquot to 50 milliliters with buffer and proceed as directed in § 440.80a(b)(1) of this chapter to determine the number of units per milliliter of this solution, and from this value calculate the number of units per milliliter of the drug. The content of buffered crystalline penicillin is satisfactory if it is not less than 85 percent of that which it is represented to contain.

(c) *Benzathine penicillin G content.* The benzathine penicillin G content of the batch is the difference between the total potency as described in paragraph (a) or (d) of this section and the content of buffered crystalline penicillin determined by the method prescribed in paragraph (b) of this section. The content of benzathine penicillin G is satisfactory if it is not less than 85 percent of that which it is represented to contain.

(d) *Total potency of a single-dose container.* Add sufficient distilled water to the material remaining in the 10-milliliter volumetric flask referred to in paragraph (b) of this section to bring the volume back to 10 milliliters and determine the number of units per milliliter of this suspension. If the iodometric method is used, 2.0-milliliter aliquots are placed in 50-milliliter volumetric flasks (one blank and one to be inactivated). Obtain the total potency by adding the number of units found in the 10-milliliter volumetric flask to one-half the content of buffered crystalline penicillin found in paragraph (b) of this section.

(e) *Sterility.* Proceed as directed in § 436.20 using the method described in paragraph (e)(2) of that section, except use medium C in lieu of medium A, and medium F in lieu of medium E. During the period of incubation, shake the tubes at least once daily.

(f) *Moisture.* Proceed as directed in § 440.74a(b)(5) of this chapter.

(g) *Pyrogens.* Proceed as directed in § 436.500.

(h) *Toxicity.* Proceed as directed in § 440.55a(b)(3) of this chapter.

(i) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using the suspension resulting when the product is reconstituted as directed in the labeling.

§ 436.507 Benzathine-procaine-buffered crystalline penicillins for aqueous injection.

(a) *Potency*—(1) *Total potency.* Proceed as directed in § 440.80a(b)(1) of this chapter, except if the bioassay method is used prepare the sample by diluting one dose of the drug suspension with sufficient dimethyl formamide or formamide or methyl alcohol to dissolve the benzathine penicillin G. Make to 100 milliliters with 1-percent phosphate buffer, pH 6.0. Shake well, and dilute to 1.0 unit per milliliter with buffer. If the iodometric method of assay is used, add the indicated amount of distilled water to the contents of a vial of the sample, shake well, and proceed as follows (except for single-dose containers):

(i) Using a standardized hypodermic syringe, withdraw one dose and dilute with 1-percent phosphate buffer, pH 6.0, to give a concentration of approximately 2,000 units per milliliter. Use 2.0 milliliters of this suspension as the blank in the iodometric assay procedure described in § 440.80a(b)(5)(i)(*a*) of this chapter.

(ii) Using a standardized hypodermic syringe, withdraw another dose, place in a flask, and add 20 milliliters of 0.5 *N* NaOH for each 300,000 units of benzathine penicillin, mix well, being sure that all penicillin is in solution, and allow to stand for 15 minutes. Add 1 milliliter of 1.2 *N* HCl for each 2 milliliters of 0.5 *N* NaOH, mix, and dilute with distilled water to the same volume as was used in paragraph (a)(1)(i) of this section. Place 2.0 milliliters in a 125-milliliter glass-stoppered Erlenmeyer flask, add 10 milliliters of 0.01 *N* iodine, allow to stand for 15 minutes, and titrate with 0.01 *N* sodium thiosulfate as directed in the iodometric assay procedure in § 440.80a(b)(5)(i)(*a*) of this chapter. The total potency of the batch is satisfactory if it contains not less than 85 percent of that which it is represented to contain.

(2) *Procaine penicillin content (except for single-dose containers).* Make suitable dilutions of the solution prepared in paragraph (a)(1)(ii) of this section to obtain approximately 60 units of procaine penicillin per milliliter. Determine the procaine penicillin content by the colorimetric procedure described in § 436.503(b)(3). The content of procaine penicillin is satisfactory if it contains not less than 85 percent of the number of units that it is represented to contain.

(3) *Buffered crystalline penicillin content*—(i) *Preparation of the solution for assay.* (*a*) Add the indicated amount of distilled water to the contents of a vial of the sample, and shake well. Withdraw one dose of the suspension with a hypodermic syringe and place in a 10-milliliter volumetric flask. Add 20-percent sodium sulfate solution almost to the mark, centrifuge sufficiently to see the meniscus, make to volume with 20-percent sodium sulfate solution, shake well, and centrifuge to obtain a clear or reasonably clear solution; or

(*b*) If the original product contains more than 600,000 units, place it in a 50-milliliter volumetric flask, add 20-percent sodium sulfate to the mark, shake well, place a 10-milliliter portion in a centrifuge tube, and centrifuge to obtain a reasonably clear solution.

(c) Dilute a 5.0-milliliter aliquot of the clear solution obtained in paragraph (a)(3)(i)(*a*) or (*b*) of this section with 1-percent phosphate buffer, pH 6.0, to give a solution for assay of approximately 2,000 units per milliliter.

(ii) *Iodometric assay for total penicillin in the solution for assay.* Determine the total quantity of penicillin in the solution for assay by the iodometric assay procedure described in § 440.80a(b)(5)(iv)(*a*) of this chapter.

(iii) *Colorimetric determination of procaine penicillin in the solution for assay.* Proceed as directed in § 436.503(b)(3). The content of procaine penicillin in the batch is satisfactory if it is not less than 85 percent of that which it is represented to contain.

(iv) The buffered crystalline penicillin in one dose of the product is calculated as follows:

$$A = (B - C)F,$$

where

A = the buffered crystalline penicillin content of the product.

B = the number of units of penicillin per milliliter as determined in paragraph (a)(3)(ii) of this section.

C = the number of units of procaine penicillin per milliliter as determined in paragraph (a)(3)(iii) of this section.

F = the appropriate dilution factor depending on the dilutions made in the preparation of the solution for assay.

The content of buffered crystalline penicillin is satisfactory if the batch contains 85 percent of the number of units per milliliter that it is represented to contain.

(4) *Benzathine penicillin content.* The sum of the procaine penicillin content determined under paragraph (a)(2) or (6) of this section and the buffered crystalline penicillin content determined under paragraph (a)(3) of this section, subtracted from the total potency determined in paragraph (a)(1) or (5) of this section, represents the benzathine penicillin G content. The benzathine penicillin G content is satisfactory if it is not less than 85 percent of the number of units that it is represented to contain.

(5) *Total potency of a single-dose container.* Wash out the material remaining in the volumetric flask referred to in paragraph (a)(3)(i)(*b*) of this section, or combine the contents remaining in the 50-milliliter volumetric flask and in the centrifuge tube referred to in paragraph (a)(3)(i)(b) of this section. Dissolve the material by adding 10 milliliters of 1 *N* NaOH for each 300,000 units of benzathine penicillin and allow to stand 15 minutes. Add 1 milliliter of 1.2 *N* HCl for each milliliter of 1 *N* NaOH and then dilute with distilled water to give a concentration of approximately 2,000 units per milliliter. Place 2.0 milliliters in a 125-milliliter glass-stoppered Erlenmeyer flask, add 10 milliliters of 0.01 *N* iodine, allow to stand for 15 minutes, and then

titrate with 0.01 *N* sodium thiosulfate as directed in § 440.80a(b)(5)(iv)(*a*) of this chapter. For the blank determination prepare a separate sample as directed in paragraph (a)(3)(i)(*a*) or (*b*) of this section and in the first sentence of this paragraph (a)(5), then dilute with 1 percent phosphate buffer, pH 6.0, to give a concentration of approximately 2,000 units per milliliter. The total potency of the one-dose container is equal to the sum of the number of units found in this assay (units per milliliter×volume) and the number of units found (units per milliliter ×volume) in the solution for assay in paragraph (a)(3)(ii) of this section.

(6) *Procaine penicillin content of a single-dose container.* Make suitable dilutions of the NaOH-inactivated solution prepared in paragraph (a)(5) of this section to obtain approximately 60 units of procaine penicillin per milliliter. Determine the procaine penicillin content (units per milliliter× volume) of this solution by the colorimetric procedure described under § 436.503(b)(3). To this value add per procaine penicillin content (unit per milliliter×volume) of the solution for assay, as found in paragraph (a)(3)(iii) of this section, to obtain the procaine penicillin content of the one-dose container. The content of procaine penicillin in the batch is satisfactory if it is not less than 85 percent of that which it is represented to contain.

(b) *Sterility.* Proceed as directed in § 436.20, using the method described in paragraph (e)(2) of that section, except use medium C in lieu of medium A, and medium F in lieu of medium E. During the period of incubation, shake the tubes at least once daily.

(c) *Pyrogens.* Proceed as directed in § 440.55a(b)(4) of this chapter.

(d) *Toxicity.* Proceed as directed in § 440.55a(b)(3) of this chapter.

(e) *Moisture.* Proceed as directed in § 440.74a(b)(5) of this chapter.

(f) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using the suspension resulting when the product is reconstituted as directed in the labeling.

§ 436.508 Penicillin-bacitracin-neomycin ointment; penicillin-bacitracin-neomycin in oil.

(a) *Potency*—(1) *Penicillin content; bacitracin content.* Proceed as directed in § 436.504(a).

(2) *Neomycin content.* Proceed as directed in § 448.510d(b)(1)(ii) of this chapter, except that sufficient penicillinase is added to the sample under test to completely inactivate the penicillin present. Its content of neomycin is satisfactory if it contains not less than 85 percent of the number of milligrams per gram that it is represented to contain.

(b) *Moisture.* Proceed as directed in § 436.201.

§ 436.509 Procaine penicillin-streptomycin-polymyxin in oil; procaine penicillin-dihydrostreptomycin-polymyxin in oil; procaine penicillin-streptomycin-polymyxin ointment; procaine penicillin-dihydrostreptomycin-polymyxin ointment.

(a) *Potency*—(1) *Penicillin content.* Proceed as directed in § 141a.8(a) of this chapter. Its content of penicillin is satisfactory if it contains not less than 85 percent of the number of units per milliliter or per gram that it is represented to contain.

(2) *Streptomycin content.* Proceed as directed in § 141b.129(a)(1) of this chapter, except inactivate the penicillin in the combined extractives with sufficient penicillinase at 37° C. for 30 minutes. Its content of streptomycin is satisfactory if it contains not less than 85 percent of the number of milligrams per milliliter or per gram that it is represented to contain.

(3) *Dihydrostreptomycin content.* Proceed as directed in paragraph (a)(2) of this section, using the dihydrostreptomycin working standard as a standard of comparison. Its content of dihydrostreptomycin is satisfactory if it contains not less than 85 percent of the number of milligrams per milliliter or per gram that it is represented to contain.

(4) *Polymyxin content.* Proceed as directed in § 444.170a(b)(2)(i) of this chapter, with the following exceptions:

(i) In lieu of the directions for the preparation of the sample described in § 444.170a(b)(2)(i)(*g*) of this chapter, prepare the sample by one of the following techniques:

(*a*) *Extraction. Place a convenient-*sized representative quantity of the sample in a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 25 milliliters of 10-percent potassium phosphate buffer (pH 6.0) and shake. Remove the buffer layer and repeat the extraction with 25-milliliter portions of buffer at least three times and any additional times that may be neccessary to insure complete extraction of the antibiotic. Combine the extractives. Inactivate the penicillin with sufficient penicillinase at 37° C. for 30 minutes. Make the proper estimated dilutions in 10-percent potassium phosphate buffer (pH 6.0) to give a concentration of 10 units per milliliter (estimated).

(*b*) *Blending.* Place a convenient-sized representative quantity of the sample in a blending jar containing 1.0 milliliter of polysorbate 80 and sufficient 1-percent phosphate buffer (pH 6.0) to give a final volume of 200 milliliters. If the sample consists of substantially more than 1 gram, use sufficient buffer to give a final volume of 500 milliliters. If the concentration of polymyxin in the blend is less than 200 units per milliliter, 10-percent phosphate buffer (pH 6.0) should be used in lieu of 1-percent phosphate buffer (pH 6.0). Using a high-speed blender, blend the mixture for 2 minutes. Inactivate the penicillin with sufficient penicillinase at 37° C. for 30 minutes and make the proper estimated dilutions in 10-percent phosphate buffer (pH 6.0) to give a concentration of 10 units per milliliter (estimated).

(ii) The standard curve is prepared in the following concentrations: 6.4, 8.0, 10.0, 12.5, and 15.6 units per milliliter in 10-percent potassium phosphate buffer, pH 6.0. The 10 units per milliliter concentration is used as the reference point. Its content of polymyxin is satisfactory if it contains not less than 85 percent of the number of units per milliliter or per gram that it is represented to contain.

(b) *Moisture.* Proceed as directed in § 436.201.

§ 436.510 Penicillin-streptomycin-erythromycin ointment; penicillin-dihydrostreptomycin-erythromycin ointment.

(a) *Potency*—(1) *Penicillin content.* Obtain the weight of the content of a syringe by weighing before and after ejecting the content into a beaker. Stir until homogeneous. Remove a representative sample (usually approximately 1.0 gram, accurately weighed) and place in a separatory funnel containing 50 milliliters of peroxide-free ether. Add 20 milliliters of 0.1 *M* potassium phosphate buffer (pH 8.0) and shake. Remove the buffer layer and repeat the extraction with three additional 20-milliliter portions of the buffer. Place the buffer solution in a second separatory funnel and wash with three 30-milliliter portions of ether. Discard the ether washes. Remove an aliquot of the buffer solution and proceed as directed in § 440.80a(b)(1) of this chapter, except § 440.80a(b)(1)(iv) and (ix) of this chapter. If the iodometric chemical assay is used, proceed as directed in § 440.80a(b)(5)(iv)(*a*) of this chapter, except prepare the sample as directed in § 141a.35(a)(1) of this chapter. Its content of penicillin is satisfactory if it contains not less than 85 percent of the number of units that it is represented to contain.

(2) *Streptomycin content.* Using an aliquot of the buffer solution prepared as directed in paragraph (a)(1) of this section, proceed as directed in § 444.70a(b)(1) through (9) of this chapter, except add sufficient penicillinase to completely inactivate the penicillin present. Its content of streptomycin is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(3) *Dihydrostreptomycin content.* Proceed as directed in paragraph (a)(2) of this section, using the dihydrostreptomycin working standard as the standard of comparison. Its content of dihydrostreptomycin is satisfactory if it contains not less than 85 percent of the

number of milligrams that it is represented to contain.

(4) *Erythromycin content.* Proceed as directed in § 444.570b(b)(1)(i)(*b*) of this chapter, except prepare the sample as follows: Place a representative sample (usually approximately 1.0 gram, accurately weighed), in a glass blending jar containing 99 milliliters of 0.1 *M* potassium phosphate buffer, pH 8.0, and 1 milliliter of polysorbate 80. Using a high-speed blender, blend for 2 to 3 minutes. Add 100 milliliters of 0.1 *M* potassium phosphate buffer, pH 8.0, and blend for an additional 2 to 3 minutes. Prepare an intermediate dilution by diluting an aliquot of the filtrate with 0.1 *M* potassium phosphate buffer (pH 8.0), and add sufficient penicillinase to inactivate the penicillin. Then further dilute with buffer to give an erythromycin content of 1.0 microgram per milliliter (estimated). Its content of erythromycin is satisfactory if it contains not less than 85 percent of the number of milligrams that it is presented to contain.

(b) *Moisture.* Proceed as directed in § 436.500(c).

§ 436.511 Penicillin-streptomycin - bacitracin methylene disalicylate-neomycin ointment; penicillin-dihydrostreptomycin-bacitracin methylene disalicylate-neomycin ointment.

(a) *Potency*—(1) *Penicillin content.* Proceed as directed in § 141a.8(a) of this chapter. Its penicillin content is satisfactory if it contains not less than 85 percent of the number of units that it is represented to contain.

(2) *Streptomycin content.* Proceed as directed in § 141a.65(a)(2) of this chapter. Its content of streptomycin is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(3) *Dihydrostreptomycin content.* Proceed as directed in § 141a.65(a)(3) of this chapter. Its content of dihydrostreptomycin is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(4) *Bacitracin methylene disalicylate content.* Proceed as directed in § 436.505(a)(3). Its potency is satisfactory if it contains not less than 85 percent of the equivalent number of units of bacitracin that it is represented to contain.

(5) *Neomycin content.* Proceed as directed in § 141a.65(a)(4)(ii) of this chapter. Its content of neomycin is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(b) *Moisture.* Proceed as directed in § 436.201.

§ 436.512 Procaine penicillin G-novobiocin - neomycin - dihydrostreptomycin in oil.

(a) *Potency*—(1) *Penicillin G content.* Proceed as directed in §440.180d(b)(1)(i)(*a*) of this chapter, using the novobiocin-resistant strain of *Staphylococcus aureus* (ATCC 12692),[1] except prepare the sample as follows: Place the equivalent of one dose of sample in a blending jar, add 1.0 milliliter of polysorbate 80 and a quantity of 1 percent potassium phosphate buffer, pH 6.0, sufficient to make a total of 500 milliliters. Blend for 5 minutes with a high-speed blender and make appropriate dilutions, using 1 percent potassium phosphate buffer, pH 6.0. Its content of penicillin G is satisfactory if it contains not less than 85 percent of the number of units that it is represented to contain.

(2) *Novobiocin content.* Proceed as directed in § 440.180d(b)(3)(i), with the following exceptions:

(i) Prepare the sample as follows: Place the equivalent of one dose of sample in a blending jar, add 1.0 milliliter of polysorbate 80 and a quantity of 0.1*M* potassium phosphate buffer, pH 8.0, sufficient to make a total of 500 milliliters. Blend for 5 minutes with a high-speed blender. To an aliquot, add sufficient penicillinase to inactivate the penicillin, further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6) to give a final concentration of 0.5 microgram novobiocin per milliliter (estimated), and allow to stand for ½-hour at 37° C. before filling the plates.

(ii) Aseptically add to the seed agar used for this assay, at the time the bacterial suspension is added, a slurry of Dowex 50 WX–4, Na^+ type 200–400 mesh, sufficient to make a total concentration of 2 percent. Prepare the slurry by adding 50 grams of the resin to 30 milliliters of distilled water and sterilize for 15 minutes at 15 pounds pressure. Mix the slurry thoroughly before adding. Its content of novobiocin is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(3) *Neomycin content.* Proceed as directed in § 436.517(b)(1) of this chapter, using the *Staphylococcus epidermidis* (ATCC 12228) [1] procedure, except:

(i) Prepare the sample as follows: Place the equivalent of one dose of sample in a blending jar, add 1.0 milliliter of polysorbate 80 and a quantity of 0.1*M* potassium phosphate buffer, pH 8.0, sufficient to make a total of 500 milliliters. Blend for 5 minutes with a high-speed blender. To an aliquot, add sufficient penicillinase to inactivate the penicillin, further dilute with 0.1*M* potassium phosphate buffer, pH 8.0, to give a final concentration of 1.0 microgram neomycin per milliliter (estimated), and allow to stand for ½-hour at 37° C. before filling the plates.

(ii) Aseptically add to the seed agar used for this assay, at the time the bacterial suspension is added, a slurry of Dowex 1–X8, Cl type 200–400 mesh, to make a total concentration of 1 percent. Prepare the slurry by adding 50 grams of the resin to 30 milliliters of distilled water and sterilize for 15 minutes at 15 pounds pressure. Mix the slurry thoroughly before adding. Its content of neomycin is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(4) *Dihydrostreptomycin content.* Proceed as directed in § 141a.65(a)(3), except prepare the sample by placing the equivalent of one dose in a blender, add 1.0 milliliter of polysorbate 80 and a quantity of 0.1*M* potassium phosphate buffer, pH 8.0, sufficient to make a total of 500 milliliters. Blend for 5 minutes with a high-speed blender. To an aliquot, add sufficient penicillinase to inactivate the penicillin, further dilute with 0.1*M* potassium phosphate buffer, pH 8.0, to give a final concentration of 1.0 microgram dihydrostreptomycin per milliliter (estimated), and allow to stand for ½-hour at 37° C. before filling the plates. Its content of dihydrostreptomycin is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(b) *Moisture.* Proceed as directed in § 436.500(c).

§ 436.513 Chlortetracycline troches; tetracycline hydrochloride troches.

(a) *Potency.* If it is tetracycline hydrochloride proceed as directed in § 446.81a(b)(1) of this chapter and if it is chlortetracycline hydrochloride troches proceed as directed in § 446.10a(b)(1) of this chapter, except § 446.10a(b)(1)(x), and in lieu of the directions in § 446.10a(b)(1)(iv) and (viii)(*c*) of this chapter prepare the sample as follows: Place 12 troches in a glass blending jar containing 500 milliliters of 0.01*N* HCl. Using a high-speed blender, blend for 3 to 5 minutes and then make the proper estimated dilutions in the buffer solution. The average potency of the troches is satisfactory if they contain not less than 85 percent of the number of milligrams they are represented to contain.

(b) *Moisture.* Proceed as directed in § 440.80a(f)(5)(i) of this chapter.

§ 436.514 Chlortetracycline hydrochloride powder topical; tetracycline hydrochloride powder topical.

(a) *Potency*—(1) *Dry powder.* Using a 3.0-gram sample or the entire contents of the immediate container for each determination, prepare the sample as follows: Using a high-speed blender, blend a 3.0-gram sample in a glass blending jar containing 500 milliliters of 0.01 *N* HCl (use 0.1 *N* HCl if it is tetracycline), or reconstitute in the immediate container as directed in the labeling of the drug. Transfer an appropriate aliquot of 1.0 milliliter to 5.0 milliliters to a 100-milliliter volumetric flask and make to mark with 0.01 *N* HCl (use 0.1 *N* HCl if it is tetracycline). Withdraw an aliquot from the volumetric flask, and if it is chlortetracycline hydrochloride dilute to 0.06 μg. per milliliter, using 0.1 *M* potassium phosphate buffer, pH 4.5, and proceed as directed in § 446.10a(b)(1) of this chapter. If it is tetracycline hydrochlorida, dilute to 0.24 μg. per milliliter, using 0.1 *M* potassium phosphate buffer, pH 4.5, and proceed as directed in

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

§ 446.81a(b)(1) of this chapter. The average potency is satisfactory if it contains not less than 85 percent of the number of milligrams of chlortetracycline hydrochloride or tetracycline hydrochloride per gram or per immediate container that it is represented to contain.

(2) *Powder packaged with inert gases.* Spray, as directed in the labeling, the entire contents of each container to be tested into a separate 2-liter Erlenmeyer flask, held in a horizontal position. Add 500 milliliters of 0.1 *N* HCl and shake to dissolve the contents. Immediately remove aliquots of this solution and, using 0.1 *M* potassium phosphate buffer, pH 4.5, for further dilutions, proceed as directed in § 446.10a(b)(1) of this chapter if it is chlortetracycline hydrochloride powder or § 446.81a(b)(1) of this chapter if it is tetracycline hydrochloride powder. Calculate the average total amount of antibiotic expelled from the containers. The total potency is satisfactory if it contains not less than 85 percent of the number of milligrams of chlortetracycline hydochloride or tetracycline hydrochloride that it is represented to contain.

(b) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) of this chapter, except if it is packaged with inert gases proceed as directed in § 141b.117 (c) of this chapter.

§ 436.515 Capsules tetracycline and oleandomycin phosphate; capsules tetracycline and troleandomycin; capsules tetracycline hydrochloride and oleandomycin phosphate; capsules tetracycline hydrochloride and troleandomycin.

(a) *Potency*—(1) *Tetracycline or tetracycline hydrochloride content by turbidimetric assay*—(i) *Test culture and media.* Maintain the test organism *Escherichia* coli (ATCC 10536)[1] on the agar described in § 440.80a(b)(1)(ii)(*a*) of this chapter. For use in the assay, prepare a suspension of the organism every 2 weeks, as follows: Transfer the organism to a fresh agar slant and incubate at 37° C. overnight. Wash the growth from the slant with the aid of 2 milliliters of sterile distilled water and sterile glass beads into a Roux bottle containing 300 milliliters of the maintenance medium. Incubate overnight at 37° C. and then harvest the growth with 50 milliliters of sterile distilled water and sterile glass beads. Standardize this suspension by determining the dilution that will permit 40-percent light transmission in a photoelectric colorimeter using a 650-millimicron filter and an 18-millimeter diameter test tube as an absorption cell. Prepare the daily inoculum by adding 10 milliliters of that dilution to each liter of nutrient broth, prepared as directed in § 440.80a(b)(1)(ii)(*c*) of this chapter, needed for the test.

(ii) *Working standard and solutions.* Dissolve an appropriate amount of the working standard in sufficient 0.1 *N* HCl to give a concentration of 1,000 micrograms per milliliter. This stock solution may be kept in the refrigerator for 1 week. Make daily dilutions of the stock solution with 0.1 *M* potassium phosphate buffer (pH 4.5) to obtain concentrations of 0.146, 0.187, 0.240, 0.308, and 0.395 micrograms per milliliter. Add 1.0 milliliter of each such concentration to each of three 16 millimeters x 125 millimeters test tubes.

(iii) *Preparation of sample.* Dissolve the contents of a representative number of capsules in sufficient 0.1 *N* HCl to give a stock solution of convenient concentration. Further dilute the stock solution with 0.1 *M* potassium phosphate buffer (pH 4.5) to obtain a final concentration of 0.24 microgram per milliliter (estimated). Add 1.0-milliliter of this dilution to each of three 16 millimeters x 125 millimeters test tubes.

(iv) *Procedure.* To each of the 16 millimeters x 125 millimeters test tubes prepared in paragraph (a)(1)(ii) and (iii) of this section, add 9.0 milliliters of the inoculated nutrient broth described in paragraph (a)(1)(i) of this section and place immediately in a 37° C. water bath for 3 to 4 hours. After incubation, add 0.5 milliliter of a 12-percent formaldehyde solution to each tube and read the absorbance values in a suitable photoelectric colorimeter using a wavelength of 530 millimicrons. Set the instrument at zero absorbance with clear uninoculated broth prepared as described in § 440.80a(b)(1)(ii)(*c*) of this chapter.

(v) *Estimation of potency.* Plot the average values for each concentration of the standard on arithmetic graph paper with absorbance values on the ordinate and tetracycline or tetracycline hydrochloride concentrations on the abscissa. Construct the best straightline through the points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where

L=absorbance value for the lowest concentration of the standard curve.

H=absorbance value for the highest concentration of the standard curve.

a, b, c, d, e=average absorbance values for each concentration of the standard curve.

Plot the values obtained for L and H and connect the points with a straight line. Average the absorbance values for the sample and read the tetracycline or tetracycline hydrochloride concentration from the standard curve. Multiply the concentration by appropriate dilution factors to obtain the tetracycline or tetracycline hydrochloride content of the sample. Its potency is satisfactory if it contains the equivalent of not less than 85 percent of the number of milligrams of tetracycline hydrochloride that it is represented to contain.

(2) *Oleandomycin content.* (i) If oleandomycin phosphate is used, proceed as directed in paragraph (c)(1) of this section, except prepare the sample as follows: Dissolve the contents of a representative number of capsules in sufficient 0.1 *M* potassium phosphate buffer (pH 8.0) to give a stock solution of convenient concentration. Further dilute with 0.1 *M* potassium phosphate buffer (pH 8.0) to obtain a final concentration of 5.0 μg. of oleandomycin activity per milliliter (estimated).

(ii) If troleandomycin is used, proceed as follows: Dissolve the contents of a representative number of capsules in chloroform to give a stock solution of 1.0 milligram of oleandomycin activity per milliliter. Transfer 30 milliliters of the chloroform solution to a glass-stoppered test tube (200 millimeters x 22 millimeters) and add 20 milliliters of 1 *N* sodium hydroxide. Shake for 1 minute and centrifuge briefly to aid in the separation of the layers. With the aid of a syringe and needle, remove and discard the aqueous layer. Repeat the washing procedure with two more 20-milliliter portions of 1 *N* sodium hydroxide solution. Filter the chloroform layer through a pledget of cotton. Dilute an aliquot of this solution with chloroform to give a solution containing approximately 25 μg. of oleandomycin per milliliter. Transfer a 5.0 milliliter aliquot to a 40 milliliter glass-stoppered centrifuge tube, dilute to 20 milliliters, with chloroform, and determine the oleandomycin content as directed in paragraph (d)(1)(i) of this section.

Its content of oleandomycin is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(b) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) of this chapter.

(c) *Oleandomycin phosphate used in making the capsules*—(1) *Potency*—(i) *Cylinders (cups).* Used cylinders described in § 440.80a(b)(1)(i) of this chapter.

(ii) *Culture media.* (*a*) Use the nutrient agar described in § 440.80a(b)(1)(ii)(*a*) of this chapter for the seed layer and base layer, except that its pH after sterilization is 7.8 to 8.0.

(*b*) Use the nutrient agar described in § 440.80a(b)(1)(ii)(*a*) of this chapter for maintaining the test organism.

(iii) *Working standard.* Dissolve a suitable weighed quantity (usually 25 milligrams or less) of the working standard (obtained from the Food and Drug Administration) in 2 milliliters of ethanol, then add sufficient 0.1 *M* potassium phosphate buffer, pH 8.0, to give a concentration of 1,000 micrograms of oleandomycin base per milliliter. This stock solution may be kept in the refrigerator for 3 days.

(iv) *Preparation of sample.* Dissolve the sample in sufficient 0.1 *M* potassium phosphate buffer (pH 8.0) to give a convenient stock solution. Further dilute in 0.1 *M* potassium phosphate buffer (pH 8.0) to give a final concentration of 5.0 micrograms per milliliter (estimated).

(v) *Preparation of test organism.* The test organism is *Staphylococcus epidermidis* (ATCC) 12228,[1] which is main-

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

tained on slants or agar described under paragraph (c)(1)(ii) (*a*) of this section. Wash the organism from the agar slant with 3 milliliters of sterile physiological saline solution onto a large agar surface such as that provided by a Roux bottle containing 300 milliliters of the agar described in paragraph (c)(1)(ii) (*a*) of this section. Spread the suspension of organisms over the entire agar surface with the aid of sterile glass beads. Incubate for 4 hours at 32° C. and then wash the resulting growth from the agar surface with about 30 milliliters of sterile physiological saline solution. Standardize the suspension by determining the dilution that will give 80-percent light transmission, using a suitable photoelectric colorimeter with a 650-millimicron filter and an 18-millimeter-diameter test tube as an absorption cell. Run test plates to determine the quantity of the diluted suspension (usually 1.5 milliliters) that should be added to each 100 milliliters of agar to give clear, sharp zones of inhibition of appropriate size.

(vi) *Preparation of plates.* Add 21 milliliters of the agar prepared as described in paragraph (c)(1)(ii) (*a*) of this section to each Petri dish (20 millimeters x 100 millimeters). Distribute the agar evenly in the plates and allow it to harden. Use the plates the same day they are prepared. Melt a sufficient amount of the agar described in paragraph (c)(1)(ii) (*a*) of this section, cool to 48° C., add the proper amount of the test organism as described in paragraph (c)(1)(v) of this section and mix thoroughly. Add 4 milliliters of this inoculated agar to each Petri dish. Distribute the agar evenly in the plates, cover with porcelain covers glazed on the outside, and allow to harden. After the agar has hardened, place 6 cylinders on the agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius.

(vii) *Standard curve.* Prepare the daily standard curve by further diluting the 1,000 micrograms per milliliter stock solution in 0.1 *M* potassium phosphate buffer (pH 8.0) to obtain concentrations of 3.2, 4.0, 5.0, 6.25 and 7.80 micrograms per milliliter. Use 3 plates for the determination of each point on the curve, except the 5.0 micrograms per milliliter concentration, a total of 12 plates. On each of 3 plates fill 3 cylinders with the 5.0 micrograms per milliliter standard, and the other 3 cylinders with the concentration under test. Thus, there will be 36 five-microgram determinations and 9 determinations for each of the other points on the curve. After incubation read the diameters of the circles of inhibition in the plates. Average the readings of the 5.0 micrograms per milliliter concentration and the readings of the point tested for each set of 3 plates and average also all 36 readings of the 5.0 micrograms per milliliter concentration. The average of the 36 readings of the 5.0 micrograms per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 5.0 micrograms per milliliter reading for that set of 3 plates were the same as the correction point. Thus, if in correcting the 4.0-microgram concentration, the average of the 36 readings of the 5.0-microgram concentration were 20.0 millimeters, and the average of the 5.0-microgram concentration of this set of 3 plates were 19.8 millimeters, the correction would be +0.2 millimeter. If the average reading of the 4.0-microgram concentration of these same 3 plates were 19.0 millimeters, the corrected value would be 19.2 millimeters. Plot these corrected values, including the average of the 5.0 micrograms per milliliter concentration, on 2-cycle semilog paper, using the concentration in micrograms per milliliter as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

L = corrected zone diameter for the lowest concentration of the standard curve,

H = corrected zone diameter for the highest concentration of the standard curve,

c = average zone diameter of 36 readings of the 5.0 micrograms per milliliter standard,

a, b, d, e = corrected average values for the 3.2, 4.0, 6.25, and 7.81 micrograms per milliliter standard solutions, respectively.

Plot the values obtained for L and H and connect with a straight line.

(viii) *Assay.* Use 3 plates for each sample. Fill 3 cylinders on each plate with the standard 5.0 micrograms per milliliter solution and 3 cylinders with the 5.0 micrograms per milliliter (estimated) sample, alternating standard and sample. Incubate all plates, including those containing the standard curve, at 32° C.–35° C. overnight, and measure the diameter of each circle of inhibition. To estimate the potency of the sample, average the zone readings of the standard and the zone readings of the sample on the 3 plates used. If the sample gives a larger zone size than the average of the standard, add the difference between them to the 5.0 micrograms per milliliter zone on the standard curve. If the average sample value is lower than the standard value, subtract the difference between them from the 5.0 micrograms per milliliter value on the curve. From the standard curve, read the potencies corresponding to these corrected values of zone sizes.

(2) *Toxicity.* Proceed as directed in § 440.80a(b)(4) of this chapter, except use physiological salt solution as the diluent, and inject 0.5 milliliter of a solution containing 8 milligrams per milliliter.

(3) *Moisture.* Proceed as directed in § 440.80(b)(5)(i) of this chapter.

(4) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using a solution containing 100 milligrams per milliliter.

(5) *Crystallinity.* Proceed as directed in § 440.80a(b)(5)(iii) of this chapter.

(d) *Troleandomycin used in making the capsules*—(1) *Potency*—(i) *Chemical method*—(*a*) *Reagents and equipment.* (*1*) Methyl orange reagent: Shake 0.5 *M* boric acid solution for about 12 hours (to insure saturation) with an excess of methyl orange indicator. An alternative method is to heat the mixture to about 50° C. and shake for about an hour. Then allow to cool. Filter the saturated dye solution and wash three times with chloroform. Store the dye solution over chloroform.

(*2*) Acid-alcohol solution: Add 2 milliliters of concentrated sulfuric acid to 98 milliliters of absolute methyl alcohol.

(*3*) Glycerin: Reagent grade.

(*4*) Centrifuge tubes: 40 milliliters, glass-stoppered.

(*b*) *Procedure.* Prepare a chloroform solution containing 50.0 milligrams activity of standard oleandomycin base in 200 milliliters of solution. Transfer 10.0 milliliters of the solution to a 100-milliliter volumetric flask and dilute to volume with chloroform. Transfer 2.0, 4.0, 6.0, and 8.0 milliliters of this solution to glass-stoppered centrifuge tubes (40-milliliter size) and dilute to a total volume of 20.0 milliliters each with chloroform. To the 20.0 milliliters of the solution present in each (40-milliliter size) centrifuge tube add 0.2 milliliter of glacial acetic acid, 0.20 milliliter of glycerin, and 0.40 milliliter of methyl orange reagent. Shake for 5 minutes and centrifuge for 3 minutes. Immediately transfer to another tube a 10.0-milliliter aliquot from the chloroform (lower) layer. Care must be exercised to see that no portion of the dye-glycerin-phase is included with the chloroform aliquot. Add 1.0 milliliter of acid-alcohol solution to this chloroform aliquot, mix well, and read the absorbancy at 535 mμ, using a 1-centimeter cell and a suitable photometer and using chloroform, similarly treated, as a blank. Prepare a standard curve, plotting the absorbance values of the standard solutions against the concentration expressed in micrograms per aliquot. Accurately weigh the sample to be tested to give 50 milligrams (estimated) of oleandomycin activity, dissolve in chloroform, and make to 200 milliliters with chloroform. Transfer 10.0 milliliters to a 100-milliliter volumetric flask and make to volume with chloroform. Transfer 5.0 milliliters to a glass-stoppered centrifuge tube and proceed as above. Determine the potency of the sample from the standard curve.

(ii) *Microbiological assay.* Proceed as directed in paragraph (c)(1) of this section, except:

(*a*) In lieu of the directions in paragraph (c)(1)(ii)(*a*) of this section, use the nutrient agar described in § 440.80a(b)(1)(ii)(*a*) of this chapter for the seed and base layers, except add 2.0 milliliters of polysorbate 80 to each 100 milliliters of agar. Its pH after sterilization is 7.8 to 8.

(b) In lieu of the directions in paragraph (c)(1)(iii) of this section, dissolve a suitable weighed quantity (usually 25 milligrams or less) of the troleandomycin working standard (obtained from the Food and Drug Administration) in sufficient 80 percent isopropyl alcohol-water solution to give a concentration of 1,000 micrograms per milliliter (estimated). Use the solution the day that it is prepared.

(c) In lieu of the directions in paragraph (c)(1)(iv) of this section, dissolve the sample in sufficient 80 percent isopropyl alcohol-water solution to give a convenient stock solution. Further dilute in 0.2 *M* potassium phosphate buffer, pH 10.5 (35 grams of dipotassium phosphate plus 2 milliliters of 10 *N* NaOH, q.s. to 1 liter), to give a final concentration of 15 micrograms per milliliter (estimated).

(d) In lieu of the directions in paragraph (c)(1)(vi) of this section, use the agar described in paragraph (d)(1)(ii)(*a*) of this section for both layers. Use the plates as soon after seeding as is practical. If they are not to be used shortly after seeding, then they should be refrigerated until ready for use.

(e) In lieu of the directions for preparing the standard curve in paragraph (c)(1)(vii) of this section, prepare the standard curve by diluting the stock solution in 0.2 *M* potassium phosphate buffer, pH 10.5, to give concentrations of 9.6, 12.0, 15.0, 18.8, and 23.4 micrograms per milliliter. The 15.0 micrograms per milliliter is the reference concentration.

(f) In lieu of the directions in paragraph (c)(1)(viii) of this section, incubate the plates at 37° C. overnight. The concentration of the sample and standard being tested is 15.0 micrograms per milliliter.

(2) *Toxicity.* Administer orally, by means of a cannula or other suitable device, to each of five mice within the weight range of 18 grams to 25 grams, 0.5 milliliter of a suspension containing 200 milligrams per milliliter in normal saline solution. If no animal dies within 48 hours, the sample is nontoxic. If one or more animals die within 48 hours, repeat the test, using for each test five or more previously unused mice weighing 20 grams (±0.5 gram) each; if the total deaths within 48 hours is no greater than 10 percent of the total number of animals tested, including the original test, the sample is nontoxic.

(3) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) of this chapter.

(4) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using a saturated aqueous-ethanol (1:1) solution prepared by adding 100 milligrams per milliliter.

(5) *Paper chromatograph method*—(i) *Apparatus and reagents*—(*a*) Chromatographic chamber (cylinder glass-stoppered museum jar 11.5 inches x 3.5 inches).

(b) Chromatographic paper (8 inches x 8 inches Whatman No. 1).

(c) 0.1 *N* hydrochloric acid.

(d) Resolving solvent: Butyl acetate, benzene, nitromethane, pyridine (5:5:5:1 by volume).

(e) Spray reagent: 15 grams antimony trichloride per 100 milliliters of chloroform.

(ii) *Procedure.* Dissolve the sample in chloroform to give a solution containing 10 milligrams to 20 milligrams per milliliter. Prepare a sheet of chromatographic paper by drawing a line of origin parallel to and 1 inch from the edge of the paper. Wet the paper thoroughly with the 0.1 *N* hydrochloric acid and blot it firmly between sheets of absorbent paper. Starting 2 inches in from the edges and at 1-inch intervals, apply 3 microliters to 5 microliters of the sample solutions to the starting line. Allow a few minutes for the paper to dry partially. While the paper is still damp, form a cylinder by bringing the outer edges together, allowing about 1-inch overlap, and secure with a paper clip. Stand the paper in the chromatographic chamber, which has been filled to a depth of ½-inch with the resolving solvent. After the solvent front rises to a height of 4 inches to 5 inches above the origin, remove the paper from the tank and hang it up to air dry. Spray the dried paper with the antimony trichloride reagent. Hang the paper in a 100° C. oven for 3 minutes. A purple spot becomes visible for trioleandomycin at an R' value of about 0.85. The approximate R' values for diacetyloleandomycin, monoacetyloleandomycin, and oleandomycin are, respectively, 0.72, 0.27, and 0.13.

(6) *Acetyl determination*—(i) *Apparatus and reagents.* (*a*) One three-necked Pyrex flask of approximately 45 milliliters capacity, pear-shaped with T-joints, agar inlet tube, glass-stoppered funnel, glass condenser, and bubble counter.

(b) 50-milliliter Pyrex Erlenmeyer flask.

(c) 10-milliliter burette, calibrated in 0.02 milliliter.

(d) Anhydrous methanol, reagent grade.

(e) 2 *N* sodium hydroxide solution.

(f) Sulfuric acid solution prepared by adding 100 milliliters of concentrated H_2SO_4 to 200 milliliters of water.

(g) 1 *N* barium chloride solution.

(h) Phenolphthalein solution (1 percent in ethanol).

(i) Water-pumped nitrogen.

(j) NaOH solution, 0.015 *N*.

(ii) *Procedure.* Weight accurately (to 0.01 milligram) approximately 30 milligrams of the sample into the three-necked acetyl flask. Add 2.0 milliliters of methanol to dissolve the sample, then add slowly with gentle swirling, 1.0 milliliter of NaOH solution. Connect the gas inlet tube with bubble counter attached, and adjust nitrogen flow to about two bubbles a second. Put glass-stoppered funnel in centerneck of acetyl flask and put about 5 milliliters of H_2O in the funnel. Add a boiling chip to the solution and attach condenser in the refluxing position with water cooling. Adjust burner flame under acetyl flask to reflux solution gently. Reflux for 30 minutes. Cool assembly slightly then rinse down condenser (still in reflux position) with a few milliliters of H_2O. Reassemble condenser to the distillation position and add water through the funnel to make a total of approximately 5 milliliters of H_2O added to acetyl flask. Adjust burner flame so that about 5 milliliters of H_2O and methanol is distilled over in approximately 10 minutes. Discard this distillate. Cool acetyl flask slightly. Acidify solution in flask by adding 1 milliliter of the sulfuric acid solution through the funnel. Adjust burner flame and distill over approximately 20 milliliters of distillate into an Erlenmeyer flask in about 20 minutes, adding water through the funnell as necessary. It is important to keep the liquid volume in the acetyl flask around 2 milliliters to 3 milliliters in order to obtain a quantitative recovery of the acetic acid. Collect a second fraction of distillate, about 10 milliliters in volume. As the second fraction is distilling, process the first fraction. Heat the first reaction and boil gently about 20 seconds. Add a few drops of $BaCl_2$ solution to check if any sulfate was distilled over. If the sulfate is present, discard and repeat the whole determination. If the sulfate is absent immediately titrate the solution with the 0.015 *N* NaOH solution to a faint pink endpoint, using one droy of phenolphthalein solution as the indicator. Repeat the above procedure with the second fraction. If the second fraction requires less than 0.10 milliliter of the 0.015 *N* NaOH solution and all the acetic acid has been distilled over, the determination is completed. If greater than this, collect a third fraction of approximately 10 milliliters and titrate this as before. Total volumes of NaOH used and calculate results as follows:

$$\frac{\text{Milliliters of NaOH} \times N \text{ NaOH} \times 0.043 \times 100}{\text{Weight sample in grams}} = \text{Percent acetyl.}$$

(7) *Crystallinity.* Proceed as directed in § 440.80a(b)(5)(iii) of this chapter.

§ 436.516 Tetracycline-neomycin complex powder topical; tetracycline hydrochloride-neomycin sulfate powder topical.

(a) *Potency.*—(1) *Tetracycline-neomycin complex powder*—(i) *Tetracycline content.* Proceed as directed in § 436.514(a)(2), except use water in lieu of 0.1 *N* HCl for dissolving the sample. Its tetracycline content is satisfactory if it contains not less than 85 percent of the equivalent number of milligrams of tetracycline hydrochloride that it is represented to contain.

(ii) *Neomycin content.* Using 0.1 *M* potassium phosphate buffer, pH 8.0, dilute an appropriate aliquot of the aqueous solution, prepared as directed in paragraph (a)(1) of this section, to a final concentration of 1 μg. per milliliter (estimated), and proceed as directed in § 436.515 (c)(1), except that the neomycin standard stock solution de-

scribed § 436.517 (b) (1) (iii) is used to prepare the standard curve, by further diluting with pH 8.0 buffer to final concentrations of 0.64, 0.80, 1.0, 1.25, and 1.56 μg. per milliliter. The 1.0 μg per milliliter solution is the reference concentration. In lieu of the method described in this subparagraph, the neomycin content may also be determined as follows. Using the aqueous solution described, prepare the sample and proceed as directed in § 436.517(b) (1), except use *Staphylococcus aureus* (American Type Culture Collection 12715)[1] as the test organism, which is grown and maintained on agar containing 100 μg. of tetracycline hydrochloride per milliliter of agar. Its neomycin content is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(2) *Tetracycline hydrochloride-neomycin sulfate powder*—(i) *Tetracycline hydrochloride content.* Prepare the sample as directed in § 436.514(a) (2). Use an appropriate aliquot and proceed as directed in § 446.81a(b) (1) of this chapter. Its tetracycline hydrochloride content is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(ii) *Neomycin content.* Use an appropriate aliquot of the solution prepared in paragraph (a) (2) (i) of this section and proceed as directed in paragraph (a) (1) (ii) of this section. Its neomycin content is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(b) *Sterility.* Thoroughly cleanse with a suitable disinfectant the valve (do not flame) of each container to be tested. Into each of two empty, sterile Erlenmeyer flasks stoppered with a cotton plug, spray quantities sufficient to yield a residue of approximately the equivalent of 50 milligrams from 10 separate cans by removing the plug temporarily and using aseptic technique while spraying; allow propellant to evaporate, add 250 milliliters to 500 milliliters of diluting fluid B in lieu of diluting fluid A, and swirl the flasks to dissolve the contents. Then proceed as directed in § 436.20 of this chapter using the method described in paragraph (e) (1) of that section.

(c) *Moisture.* Proceed as directed in § 141b.117(c) of this chapter.

(d) *Tetracycline-neomycin complex used in making the drug*—(1) *Potency*—(i) *Tetracycline content.* Dissolve the sample to be tested in sufficient water to give a convenient stock solution. Using an appropriate aliquot, proceed as directed in § 446.81a(b) (1).

(ii) *Neomycin content.* Using an aliquot of the stock solution prepared as directed in paragraph (d) (1) (i) of this paragraph, proceed as directed in paragraph (a) (2) of this section, except the last sentence of that subparagraph.

(2) *Toxicity.* Proceed as directed in § 440.80a(b) (4) of this chapter using 0.5 milliliter of a solution prepared by diluting the sample with physiological sodium chloride solution to contain 200 μg. of neomycin per milliliter (estimated).

(3) *pH.* Using a 1-percent aqueous solution, proceed as directed in § 440.80a (b) (5) (ii) of this chapter.

§ 436.517 Bacitracin-neomycin tablets; zinc bacitracin-neomycin tablets; bacitracin methylene disalicylate-neomycin tablets.

(a) *Tablets*—(1) *Potency*—(i) *Bacitracin, zinc bacitracin, or bacitracin methylene disalicylate content.* Proceed as directed in § 448.110a(b) (1). Its content of bacitracin, zinc bacitracin, or bacitracin methylene disalicylate is satisfactory if it contains not less than 85 percent of the number of units per tablet that it is represented to contain.

(ii) *Neomycin content.* Place 5 tablets in a blending jar and add thereto 200 milliliters of a 500-milliliter quantity of 0.10-percent phosphate buffer pH 8.0. After blending for 1 minute with a high-speed blender, add the remainder of the buffer. Blend again for 1 minute and make the proper estimated dilutions in the buffer and proceed as directed in paragraph (b) (1) of this section. Its content of neomycin is satisfactory if it contains not less than 85 percent of the number of milligrams of activity that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 440.80a(b) (5) (i) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 440.180a(b) (3).

(b) *Neomycin use in making the tablets*—(1) *Potency*—(i) *Cylinders (cups).* Use cylinders described under § 440.80a(b) (1) (i) of this chapter.

(ii) *Culture medium.* Use the medium described in § 440.80a(b) (1) (ii) (*a*) of this chapter for both the base and seed layers, except its pH after sterilization is 7.8 to 8.0.

(iii) *Working standard.* Dry the working standard (obtained from the U. S. P. Reference Standards Committee, 46 Park Avenue, New York 16, N.Y.) for 3 hours at 60° C. and a pressure of 5 millimeters or less and weigh out a sufficient quantity to make a convenient stock solution by diluting with a 0.1 *M* potassium phosphate buffer, pH 7.8 to 8.0. The stock solution, when stored at a temperature of approximately 15° C., or less, may be used for a period not exceeding 1 month.

(iv) *Standard curve.* Using the stock solution, prepare a daily standard curve as directed in § 444.70a(b) (1) (iv) of this chapter, using solutions of the neomycin working standard in 0.1*M* potassium phosphate buffer, pH 8.0, in concentrations of 6.4, 8.0, 10.0, 12.5, and 15.6 micrograms per milliliter if the test organism *Staphylococcus aureus* (ATCC 6538P),[1] or in concentrations of 0.64, 0.80, 1.0, 1.25, and 1.56 micrograms per milliliter if the test organism is *Staphylococcus epidermidis* (ATCC 12228).[1] The 10.0 micrograms per milliliter and the 1.0 microgram per milliliter concentrations are used as the reference points.

(v) *Preparation of test organism.* The test organism is *Staphylococcus aureus* (ATCC 6538P),[1] which is maintained on agar described in § 440.80a(b) (1) (ii) (*a*) of this chapter. From a stock slant inoculate a Roux bottle containing this same agar and incubate for 24 hours at 32° C.–35° C. Wash the resulting growth from the agar surface with about 50 milliliters of sterile sodium chloride solution. Standardize this suspension by determing the dilution that will permit 80 percent light transmission through a filter at 6500 Angstrom units in a photoelectric colorimeter. The suspension may be used for 2 weeks if it is stored under refrigeration. *Staphylococcus epidermidis* (ATCC 12228),[1] which is maintained on agar as described in § 440.80a(b) (1) (ii) (*a*) of this chapter, may also be used as the test organism. From a stock slant, inoculate a Roux bottle containing this medium and incubate for 24 hours at 32° C.–35° C. Wash the resulting growth from the agar surface, using approximately 30 milliliters of sterile sodium chloride solution. Standardize the suspension by determining the dilution that will permit 80 percent light transmission through a filter of 6500 Angstrom units in a photoelectric colorimeter. The suspension may be stored for 2 weeks under refrigeration.

(vi) *Preparation of plates.* Using the agar described in subdivision (ii) of this subparagraph and approximately a 0.5 percent inoculum of the suspension described in paragraph (b) (1) (v) of this section, prepare the plates as directed in § 440.80a(b) (1) (v) of this chapter.

(vii) *Assay.* Dissolve volumetrically in 0.1 *M* potassium phosphate buffer, pH 7.8 to 8.0, the sample to be tested to make a convenient stock solution. Further dilute volumetrically this solution with 0.1 *M* potassium phosphate buffer, pH 7.8 to 8.0, to a final concentration of 10.0 micrograms (estimated) per milliliter, if the test organism is *Staphylococcus aureus* or 1.0 microgram per milliliter (estimated) if the test organism is *Staphylococcus epidermidis.*

(2) *Toxicity.* Proceed as directed in § 440.80a(b) (4) of this chapter, using 0.5 milliliter of a solution prepared by diluting the sample to approximately 200 micrograms per milliliter with physiological salt solution.

(3) *Moisture.* In an atmosphere of about 10 percent relative humidity, transfer about 100 milligrams of the finely powdered sample to a tared weighing bottle equipped with ground-glass top and stopper. Weigh the bottle and place it in a vacuum oven, tilting the stopper on its side so that there is no closure during the drying period. Dry at a temperature of 60° C. and a pressure of 5 millimeters of mercury or less for 3 hours. At the end of the drying period fill the vacuum oven with air dried by passing it through a drying agent such as sulfuric acid or silica gel. Replace the stopper and place the weighing bottle in a desiccator over a desiccating agent such as phosphorous pentoxide or silica gel, allow to cool to room temperature, and reweigh. Calculate the percent loss.

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

(4) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using a solution containing 33 milligrams per milliliter.

PART 440—PENICILLIN ANTIBIOTIC DRUGS

Subpart A—Bulk Drugs

Sec.
440.5 Ampicillin.
440.7 Ampicillin trihydrate.
440.7a Sterile ampicillin trihydrate.
440.9a Sterile sodium ampicillin.
440.11 Carbenicillin indanyl sodium.
440.13a Sterile disodium carbenicillin.
440.15 Sodium cloxacillin.
440.19 Nonsterile sodium dicloxacillin monohydrate.
440.19a Sterile sodium dicloxacillin monohydrate.
440.25 Nonsterile hetacillin.
440.29 Nonsterile potassium hetacillin.
440.29a Sterile potassium hetacillin.
440.36a Sterile sodium methicillin.
440.41 Nonsterile sodium nafcillin monohydrate.
440.41a Sterile sodium nafcillin monohydrate.
440.49 Sodium oxacillin.
440.49a Sterile sodium oxacillin.
440.51 Penicillamine.
440.53a Sterile aluminum penicillin (aluminum penicillin salt).
440.55a Sterile benzathine penicillin G (benzathine penicillin G salt).
440.57a Sterile benzathine phenoxymethyl penicillin (benzathine phenoxymethyl penicillin salt).
440.59a Sterile chloroprocaine penicillin O (penicillin O chloroprocaine salt).
440.60a Sterile dibenzylamine penicillin G salt). (dibenzylamine penicillin G
440.61a Sterile diethylaminoethyl ester penicillin G hydriodide (penicillin G diethylaminoethyl ester hydriodide).
440.63a Sterile ephedrine penicillin (penicillin ephedrine salt), ephedrine penicillin G (penicillin G ephedrine salt).
440.65a Sterile *1*-ephenamine penicillin G (penicillin G *1*-ephenamine salt).
440.66 Hydrabamine penicillin G (hydrabamine penicillin G salt).
440.69 Hydrabamine phenoxymethyl penicillin.
440.71 Phenoxymethyl penicillin.
440.73 Potassium phenoxymethyl penicillin (potassium phenoxymethyl penicillin salt).
440.74a Sterile procaine penicillin (penicillin procaine salt), procaine penicillin G (penicillin G procaine salt).
440.80a Sterile sodium penicillin (penicillin sodium, penicillin sodium salt), sterile calcium penicillin, (penicillin calcium, penicillin calcium salt), sterile crystalline penicillin (crystalline penicillin sodium, crystalline penicillin sodium salt, crystalline penicillin potassium, crystalline penicillin potassium salt, crystalline penicillin G sodium, crystalline penicillin G sodium salt, crystalline penicillin G potassium, crystalline penicillin G potassium salt, crystalline penicillin O sodium, crystalline penicillin O sodium salt, crystalline penicillin O potassium, crystalline penicillin O potassium salt).
440.81 Buffered crystalline penicillin.
440.82 Potassium phenethicillin.

Subpart B—Oral Dosage Forms

Sec.
440.105 Ampicillin oral dosage forms.
440.105a Ampicillin tablets.
440.105b Ampicillin chewable tablets.
440.105c Ampicillin capsules.
440.105d Ampicillin for oral suspension.
440.107 Ampicillin trihydrate oral dosage forms.
440.107a Ampicillin trihydrate chewable tablets.
440.107b Ampicillin trihydrate capsules.
440.107c Ampicillin trihydrate for oral suspension.
440.107d Ampicillin trihydrate-probenecid for oral suspension.
440.111 Carbenicillin indanyl sodium tablets.
440.115 Sodium cloxacillin oral dosage forms.
440.115a Sodium cloxacillin capsules.
440.115b Sodium cloxacillin for oral solution.
440.119 Sodium dicloxacillin monohydrate oral dosage forms.
440.119a Sodium dicloxacillin monohydrate capsules.
440.119b Sodium dicloxacillin monohydrate for oral suspension.
440.125 Hetacillin oral dosage forms.
440.125a Hetacillin chewable tablets.
440.125b Hetacillin for oral suspension.
440.129 Potassium hetacillin capsules.
440.141 Sodium nafcillin monohydrate oral dosage forms.
440.141a Sodium nafcillin monohydrate tablets.
440.141b Sodium nafcillin monohydrate capsules.
440.141c Sodium nafcillin monohydrate for oral solution.
440.149 Sodium oxacillin oral dosage forms.
440.149a Sodium oxacillin capsules.
440.149b Sodium oxacillin for oral solution.
440.151 Penicillamine capsules.
440.153 Aluminum penicillin tablets.
440.155 Benzathine penicillin G oral dosage forms.
440.155a Benzathine penicillin G and crystalline penicillin tablets.
440.155b Benzathine penicillin G and phenoxymethyl pencillin tablets.
440.155c Benzathine penicillin G oral suspension; benzathine penicillin G for oral suspension (benzathine penicillin G powder).
440.157 Benzathine phenoxymethyl penicillin oral suspension; benzathine phenoxymethyl penicillin for oral suspension.
440.160 Dibenzylamine penicillin and potassium penicillin powder, buffered.
440.166 Hydrabamine penicillin G oral suspension.
440.169 Hydrabamine phenoxymethyl penicillin oral dosage forms.
440.169a Hydrabamine phenovymethyl penicillin chewable tablets.
440.169b Hydrabamine phenoxymethyl penicillin oral suspension.
440.171 Phenoxymethyl penicillin oral dosage forms.
440.171a Phenoxymethyl penicillin capsules.
440.171b Phenoxymethyl penicillin for oral suspension; potassium phenoxymethyl penicillin for oral solution.
440.173 Potassium phenoxymethyl penicillin oral dosage forms.
440.173a Potassium phenoxymethyl penicillin capsules.
440.173b Potassium phenoxymethyl penicillin chewable wafers.
440.174 Procaine penicillin in oil capsules.
440.180 Penicillin oral dosage forms.
440.180a Penicillin tablets.
440.180b Penicillin-streptomycin tablets; penicillin - dihydrostreptomycin tablets.
440.180c Crystalline penicillin G capsules (crystalline penicillin G potassium capsules, crystalline penicillin G sodium capsules).
440.180d Penicillin and novobiocin capsules.
440.180e Crystalline penicillin G oral suspension, crystalline penicillin G sodium oral suspension, potassium penicillin G oral suspension.
440.180f Buffered penicillin powder, penicillin powder with buffered aqueous diluent.
440.182 Phenethicillin potassium oral dosage forms.
440.182a Phenethicillin potassium tablets.
440.182b Phenethicillin potassium for oral solution.

Subpart C—Injectable Dosage Forms

440.207 Sterile ampicillin trihydrate for suspension.
440.209 Sterile sodium ampicillin.
440.213 Sterile disodium carbenicillin.
440.219 Sodium dicloxacillin monohydrate injectable dosage forms.
440.219a Sterile sodium dicloxacillin monohydrate.
440.219b Sodium dicloxacillin monohydrate for injection.
440.229 Potassium hetacillin injectable dosage forms.
440.229a Sterile potassium hetacillin.
440.229b Potassium hetacillin for injection.
440.236 Sodium methicillin for injection.
440.241 Sodium nafcillin monohydrate for injection.
440.249 Sodium oxacillin for injection.
440.253 Aluminum penicillin in oil.
440.255 Benzathine penicillin G injectable dosage forms.
440.255a Benzathine penicillin G in oil.
440.255b Benzathine penicillin G for aqueous injection.
440.255c Benzathine penicillin G and procaine penicillin for aqueous injection.
440.259 Chloroprocaine penicillin O for aqueous injection.
440.261 Diethylaminoethyl ester penicillin G hydriodide for aqueous injection (penicillin G diethylaminoethyl ester hydriodide for aqueous injection).
440.265 *1*-Ephenamine penicillin G injectable dosage forms.
440.265a *1*-Ephenamine penicillin G in oil.
440.265b *1*-Ephenamine penicillin G for aqueous injection.
440.274 Procaine penicillin G injectable dosage forms.
440.274a Procaine penicillin G in oil.
440.274b Procaine penicillin for aqueous injection.
440.280 Penicillin injectable dosage forms.
440.280a Sterile sodium penicillin, calcium penicillin, crystalline penicillin (crystalline penicillin sodium, crystalline penicillin potassium, crystalline penicillin G sodium, crystalline penicillin G potassium, crystalline penicillin 0 sodium, crystalline penicillin 0 potassium).
440.280b Potassium penicillin G for injection.
440.280c Crystalline penicillin and bacitracin.

Sec.
440.280d Crystalline penicillin and epinephrine in oil.
440.281 Buffered crystalline penicillin.

Subparts D–E—[Reserved]

Subpart F—Dermatologic Dosage Forms

440.563 Ephedrine penicillin tablets.

AUTHORITY: Sec. 507, 59 Stat. 463, as amended (21 U.S.C. 357).

Subpart A—Bulk Drugs

§ 440.5 Ampicillin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Ampicillin is 6-[D-α-aminobenzyl] penicillin. It is a white powder. It is so purified and dried that:

(i) It contains not less than 900 micrograms and not more than 1,050 micrograms of ampicillin per milligram.

(ii) It passes the safety test.

(iii) Its loss on drying is not more than 2.0 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 3.5 and not more than 6.0.

(v) Its ampicillin content is not less than 90 percent on an anhydrous basis.

(vi) The acid-base titration concordance is such that the difference between the percent ampicillin content when determined by nonaqueous acid titration and by nonaqueous base titration is not more than six. The potency-acid titration concordance is such that the difference between the potency value divided by 10 and the percent ampicillin content of the sample determined by the nonaqueous acid titration is not more than six. The potency-base titration concordance is such that the difference between the potency value divided by 10 and the percent ampicillin content of the sample determined by the nonaqueous base titration is not more than six.

(vii) It is crystalline.

(viii) It gives a positive identity test for ampicillin.

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5 (b) of this chapter, each package shall bear on its outside wrapper or container and the immediate container the following statement, "For use in the manufacture of nonparenteral drugs only".

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, loss on drying, pH, ampicillin content, concordance, crystallinity, and identity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Assay for potency by any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient sterile distilled water to give a stock solution containing 0.1 milligram of ampicillin per milliliter (estimated). Further dilute an aliquot of the stock solution with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter.

(iii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter.

(2) *Safety.* Proceed as directed in § 436.33 of this chapter.

(3) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Ampicillin content.* Proceed as directed in § 436.213 of this chapter, using both the titration procedures described in paragraph (e)(1) and (2) of that section. Calculate the percent ampicillin content as follows:

(i) *Acid titration.*

$$\text{Percent ampicillin content} = \frac{(A-B)\ (\text{normality of lithium methoxide reagent})\ (349.4)\ (100)\ (100)}{(\text{Weight of sample in milligrams})\ (100-m)},$$

where:
A = Milliliters of lithium methoxide reagent used in titrating the sample;
B = Milliliters of lithium methoxide reagent used in titrating the blank;
m = Percent moisture content of the sample.

Calculate the difference between the potency and the ampicillin content as follows:

$$\text{Difference} = \frac{\text{Potency in micrograms per milligram}}{10} - \text{percent ampicillin content}.$$

(ii) *Base titration.*

$$\text{Percent ampicillin content} = \frac{(A-B)\ (\text{normality of perchloric acid reagent})\ (349.4)\ (100)\ (100)}{(\text{Weight of sample in milligrams})\ (100-m)},$$

where:
A = Milliliters of perchloric acid reagent used in titrating the samples;
B = Milliliters of perchloric acid reagent used in titrating the blank;
m = Percent moisture content of the sample.

Calculate the difference between the potency and the ampicillin content as follows:

$$\text{Difference} = \frac{\text{Potency in micrograms per milligram}}{10} - \text{percent ampicillin content}.$$

(6) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(7) *Identity.* Proceed as directed in § 436.211 of this chapter, using a 0.5 percent potassium bromide disc, prepared as described in paragraph (b)(1) of that section.

§ 440.7 Ampicillin trihydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Ampicillin trihydrate is the trihydrate form of D(−)α-aminobenzyl penicillin. It is so purified and dried that:

(i) It contains not less than 900 micrograms and not more than 1,050 micrograms of ampicillin per milligram on an anhydrous basis.

(ii) It passes the safety test.

(iii) Its loss on drying is not less than 12 percent and not more than 15 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 3.5 and not more than 6.0.

(v) Its ampicillin content is not less than 90 percent on an anhydrous basis.

(vi) The acid-base titration concordance is such that the difference between the percent ampicillin content when determined by nonaqueous acid titration and by nonaqueous base titration is not more than 6. The potency-acid titration concordance is such that the difference between the potency value divided by 10 and the percent ampicillin content of the sample determined by the nonaqueous acid titration is not more than 6. The potency-base titration concordance is such that the difference between the potency value divided by 10 and the percent ampicillin content of the sample determined by the nonaqueous base titration is not more than 6.

(vii) It is crystalline.

(viii) It gives a positive identity test for ampicillin trihydrate.

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5(b) of this chapter, this drug shall be labeled "ampicillin" and each package shall bear on its outside wrapper or container and the immediate container the following statement "For use in the manufacture of nonparenteral drugs only".

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, loss on drying, pH, ampicillin content, concordance, crystallinity, and identity.

(ii) Samples required: 10 packages each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient sterile distilled water to give a stock solution containing 0.1 milligram of ampicillin per milliliter (estimated). Further dilute an aliquot of the stock solution with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter.

(iii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter.

(2) *Safety.* Proceed as directed in § 436.33 of this chapter.

(3) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Ampicillin content.* Proceed as directed in § 436.213 of this chapter, using both the titration procedures described in paragraph (e)(1) and (2) of that section. Calculate the percent ampicillin content as follows:

(i) *Acid titration.*

$$\text{Percent ampicillin content} = \frac{(A-B)\ (\text{normality of lithium methoxide reagent})\ (349.4)\ (100)\ (100)}{(\text{Weight of sample in milligrams})\ (100-m)}$$

where:
A = Milliliters of lithium methoxide reagent used in titrating the sample;
B = Milliliters of lithium methoxide reagent used in titrating the blank;
m = Percent moisture content of the sample.

Calculate the difference between the potency and the ampicillin content as follows:

$$\text{Difference} = \frac{\text{Potency in micrograms per milligram}}{10} - \text{percent ampicillin content.}$$

(ii) *Base titration.*

$$\text{Percent ampicillin content} = \frac{(A-B)\ (\text{normality of perchloric acid reagent})\ (349.4)\ (100)\ (100)}{(\text{Weight of sample in milligrams})\ (100-m)},$$

where:
A = Milliliters of perchloric acid reagent used in titrating the samples;
B = Milliliters of perchloric acid reagent used in titrating the blank;
m = Percent moisture content of the sample.

Calculate the difference between the potency and the ampicillin content as follows:

$$\text{Difference} = \frac{\text{Potency in micrograms per milligram}}{10} - \text{percent ampicillin content.}$$

(6) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(7) *Identity.* Proceed as directed in § 436.211 of this chapter, using an 0.5 percent potassium bromide disc, prepared as described in paragraph (b)(1) of that section.

Effective date: § 440.7 became effective on April 15, 1974, except that the nonproprietary name designated in paragraph (a)(2) shall not be effective until new labels are printed in the normal course of business or until September 15, 1974, whichever comes first. See 39 FR 13877, April 18, 1974.

§ 440.7a Sterile ampicillin trihydrate.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Ampicillin trihydrate is the trihydrate form of D(-)α-aminobenzyl penicillin. It is so purified and dried that:

(i) It contains not less than 900 micrograms and not more than 1,050 micrograms of ampicillin per milligram on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It passes the safety test.

(v) Its loss on drying is not less than 12 percent and not more than 15 percent.

(vi) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 3.5 and not more than 6.0.

(vii) Its ampicillin content is not less than 90 percent on an anhydrous basis.

(viii) The acid-base titration concordance is such that the difference between the percent ampicillin content when determined by nonaqueous acid titration and by nonaqueous base titration is not more than 6. The potency-acid titration concordance is such that the difference between the potency value divided by 10 and the percent ampicillin content of the sample determined by the nonaqueous acid titration is not more than 6. The potency-base titration concordance is such that the difference between the potency value divided by 10 and the percent ampicillin content of the sample determined by the nonaqueous base titration is not more than 6.

(ix) It is crystalline.

(x) It gives a positive identity test for ampicillin trihydrate.

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5 (b) of this chapter, this drug shall be labeled "ampicillin."

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, safety, loss on drying, pH, ampicillin content, concordance, crystallinity, and identity.

(ii) Samples required:

(a) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(b) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay—(1) Potency.* Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive:

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient sterile distilled water to give a stock solution containing 0.1 milligram of ampicillin per milliliter. Further dilute an aliquot of the stock solution with 0.1 *M* potassium phosphate buffer, pH 8.0 (solution 3) to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter.

(iii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except in lieu of paragraph (e)(1)(i)(*a*), prepare the sample for test as follows: From each of 10 immediate containers, aseptically transfer approximately 300 milligrams of sample into a sterile 500-milliliter Erlenmeyer flask containing approximately 400 milliliters of diluting fluid D. Add at least 200,000 Levy units[*] of penicillinase. Repeat the process using 10 additional containers. Swirl both of the stoppered flasks to completely solubilize the suspension prior to filtration and proceed as directed in paragraph (e)(1)(ii) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(f) of this chapter, using a solution containing 20 milligrams of ampicillin per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this chapter.

(5) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(7) *Ampicillin content.* Proceed as directed in § 436.213 of this chapter, using both the titration procedures described in paragraph (e)(1) and (2) of that section. Calculate the ampicillin content as follows:

(i) *Acid titration.*

[*] One Levy unit of penicillinase inactivates 59.8 units of penicillin G in 1 hour at 25° C. and at a pH of 7.0 in a phosphate buffered solution of a pure alkali salt of penicillin G when the substrate is in sufficient concentration to maintain a zero order reaction.

$$\text{Percent ampicillin content} = \frac{(A-B)\ (\text{normality of lithium methoxide reagent})\ (349.4)\ (100)\ (100)}{(\text{Weight of sample in milligrams})\ (100-m)},$$

where:
A = Milliliters of lithium methoxide reagent used in titrating the sample;
B = Milliliters of lithium methoxide reagent used in titrating the blank;
m = Percent moisture content of the sample.

Calculate the difference between the potency and the ampicillin content as follows:

$$\text{Difference} = \frac{\text{Potency in micrograms per milligram}}{10} - \text{percent ampicillin content}.$$

(ii) *Base titration.*

$$\text{Percent ampicillin content} = \frac{(A-B)\ (\text{normality of perchloric acid reagent})\ (349.4)\ (100)\ (100)}{(\text{Weight of sample in milligrams})\ (100-m)},$$

where:
A = Milliliters of perchloric acid reagent used in titrating the samples;
B = Milliliters of perchloric acid reagent used in titrating the blank;
m = Percent moisture content of the sample.

Calculate the difference between the potency and the ampicillin content as follows:

$$\text{Difference} = \frac{\text{Potency in micrograms per milligram}}{10} - \text{percent ampicillin content}.$$

(8) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(9) *Identity.* Proceed as directed in § 436.211 of this chapter, using an 0.5 percent potassium bromide disc, prepared as described in paragraph (b)(1) of that section.

Effective date: § 440.7a became effective on April 15, 1974, except that the nonproprietary name designated in paragraph (a)(2) shall not be effective until new labels are printed in the normal course of business or until September 15, 1974, whichever comes first. See 39 FR 13877, April 18, 1974.

§ 440.9a Sterile sodium ampicillin.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Sterile sodium ampicillin is the sodium salt of D(-)α-aminobenzyl penicillin. It is so purified and dried that:

(i) Its potency is not less than 845 micrograms and not more than 988 micrograms of ampicillin per milligram. If it is packaged for dispensing, it contains not less than 90 percent and not more than 115 percent of the number of milligrams of ampicillin that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It passes the safety test.

(v) Its moisture content is not more than 2 percent.

(vi) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 8.0 and not more than 10.0.

(vii) Its ampicillin content is not less than 84.5 percent.

(viii) The potency-base titration concordance is such that the difference between the potency value divided by 10 and the percent ampicillin content of the sample determined by the nonaqueous base titration is not more than six.

(ix) It is crystalline.

(x) It passes the identity test for sodium ampicillin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, safety, moisture, pH, ampicillin content, concordance, crystallinity, and identity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in manufacturing another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*2*) For sterility testing: 20 packages each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 15 immediate containers or if each vial contains 250 milligrams or less of ampicillin a minimum of 24 vials.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay—*(1) *Potency—*(i) *Sample preparation.* Dissolve an accurately weighed sample in sufficient sterile distilled water to give a stock solution containing 0.1 milligram of ampicillin per milliliter (estimated), for the microbiological agar diffusion assay and in distilled water for the iodometric assay or for the hydroxylamine colorimetric assay to give a stock solution of convenient concentration; and also, if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container, or if the labeling specifies the amount of potency in a given volume of the resultant preparation remove an accurately measured representative portion from each container. Dilute with either sterile distilled water or distilled water to give a stock solution as specified above.

(ii) *Assay procedure.* Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, diluting an aliquot of the stock solution with distilled water to the prescribed concentration.

(*c*) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.22(b) of this chapter, using a solution containing 20 milligrams of ampicillin per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this chapter.

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams of ampicillin per milliliter.

(7) *Ampicillin content.* Proceed as directed in § 436.213 of this chapter, using the titration procedure described in paragraph (e)(2) of that section. Calculate the ampicillin content as follows:

$$\text{Percent ampicillin content} = \frac{(A-B)\ (\text{normality of perchloric acid reagent})\ (174.7)\ (100)\ (100)}{(\text{Weight of sample in milligrams})\ (100-m)},$$

where:

A = Milliliters of perchloric acid reagent used in titrating the sample;
B = Milliliters of perchloric acid reagent used in titrating the blank;
m = Percent moisture content of the sample.

Calculate the difference between the potency and the ampicillin content as follows:

$$\text{Difference} = \frac{\text{Potency in micrograms per milligram}}{10} - \text{percent ampicillin content}.$$

(8) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(9) *Identity.* Proceed as directed in § 436.211 of this chapter, using the method described in paragraph (b)(2) of that section.

§ 440.11 Carbenicillin indanyl sodium.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Carbenicillin indanyl sodium is the monosodium salt of *N*-(2-carboxy-3,3-dimethyl-7-oxo - 4 - thia-1-azabicyclo [3.2.0] hept-6-yl)-2-phenylmalonamic acid, 1-(5-indanyl) ester. It is so purified and dried that:

(i) Its potency is not less than 659 micrograms and not more than 769 micrograms of carbenicillin per milligram on an anhydrous basis at the time of certification, and not less than 630 micrograms of carbenicillin per milligram on an anhydrous basis at any time during the expiration period.

(ii) It passes the safety test.

(iii) Its moisture content is not more than 2.0 percent.

(iv) Its pH in an aqueous solution containing 100 milligrams per milliliter is not less than 5.0 nor more than 8.0.

(v) It gives a positive result to the identity test for carbenicillin indanyl sodium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, moisture, pH, and identity.

(ii) Samples required: Five packages, each containing approximately 1.0 gram and one package containing approximately 2.5 grams.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 436.300 of this chapter.

(2) *Safety.* Proceed as directed in § 436.33 of this chapter.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams per milliliter.

(5) *Identity.* Proceed as directed in § 436.211 of this chapter, using the 0.5-percent potassium bromide disc prepared as described in paragraph (b)(1) of that section.

§ 440.13a Sterile disodium carbenicillin.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Disodium carbenicillin is the disodium salt of α-carboxybenzylpenicillin. It is so purified and dried that:

(i) It contains not less than 770 micrograms of carbenicillin per milligram on an anhydrous basis. If it is packaged for dispensing, its carbenicillin content is not less than 90 percent and not more than 120 percent of the number of milligrams of carbenicillin that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It passes the safety test.

(v) Its moisture content is not more than 6 percent.

(vi) Its pH in an aqueous solution containing 10 milligrams of carbenicillin per milliliter (or if packaged for dispensing, after reconstitution as directed in the labeling) is not less than 6.5 and not more than 8.0.

(vii) It gives a positive identity test for disodium carbenicillin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Request for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, safety, moisture, pH, and identity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams; and 5 packages, each containing approximately 1 gram.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 15 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration; and also if it is packaged for dispensing, reconstitute as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. If it is a single dose container, use a separate needle and syringe for each container. Dilute with sufficient solution 1 to give a stock solution of convenient concentration. Further dilute the stock solution with solution 1 to the reference concentration of 20.0 micrograms of carbenicillin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 100 milligrams of carbenicillin per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this chapter.

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams of carbenicillin per milliliter (or if packaged for dispensing, use a solution prepared as directed for reconstitution in the labeling).

(7) *Identity.* Proceed as directed in § 436.211 of this chapter, using a 0.5 percent potassium bromide disc prepared as directed in paragraph (b)(1) of that section.

§ 440.15 Sodium cloxacillin.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Sodium cloxacillin is the monohydrate sodium salt of 5-methyl-3-(*o*-chlorophenyl)-4-isoxazolyl penicillin. It is so purified and dried that:

(i) Its potency is not less than 825 micrograms of cloxacillin per milligram.

(ii) It passes the safety test.

(iii) Its moisture content is not less than 3 percent and not more than 5 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 4.5 nor more than 7.5.

(v) Its sodium cloxacillin content is not less than 90 percent.

(vi) It passes the identity test.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, moisture, pH, sodium cloxacillin content, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay—*(1) *Potency.* Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration.

Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 5 micrograms of cloxacillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this subchapter.

(iii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this subchapter.

(2) *Safety.* Proceed as directed in § 436.33 of this subchapter.

(3) *Moisture.* Proceed as directed in § 436.201 of this subchapter.

(4) *pH.* Proceed as directed in § 436.202 of this subchapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Sodium cloxacillin content.* Accurately weigh approximately 100 milligrams of the sample and dissolve in sufficient 5*N* sodium hydroxide to give a total volume of 25 milliliters. Place in a boiling water bath for 30 minutes. Cool, acidify 1 milliliter with 1 milliliter of dilute sulfuric acid (1 in 2), add 8 milliliters of water, and extract with two 25-milliliter portions of ethyl ether. Combine the ether extractives and extract with 25-milliliter portions of 0.1*N* sodium hydroxide. Combine the alkaline extractives and dilute to 100 milliliters with carbon dioxide-free water. Treat a portion of the cloxacillin working standard in the same maner. Using a suitable spectrophotometer, determine the absorbance of the solution in a 1-centimeter cell at the absorption peaks at 257±3 nanometers and at 282±3 nanometers compared with a reagent blank. Determine the percent sodium cloxacillin in the sample by means of the following calculation:

$$\text{Percent sodium cloxacillin} = \frac{A_1 \times \text{weight of standard in milligrams, on an "as is" basis} \times \text{percent sodium cloxacillin in the standard}}{A_2 \times \text{weight of sample in milligrams on an "as is" basis}} \times 100$$

where:

A_1 = Difference in absorbance for the sample between 257 nanometers and 282 nanometers;

A_2 = Difference in absorbance for the cloxacillin working standard, similarly treated.

(6) *Identity.* Proceed as directed in § 436.211 of this subchapter, using the 0.5 percent potassium bromide disc described in paragraph (b)(1) of that section.

(7) *Crystallinity.* Proceed as directed in § 436.203 of this subchapter.

§ 440.19 Nonsterile sodium dicloxacillin monohydrate.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Nonsterile sodium dicloxacillin monohydrate is the monohydrated sodium salt of 5-methyl-3-(2,6-dichlorophenyl)-4-isoxazolyl penicillin. It is so purified and dried that:

(i) Its potency is not less than 850 micrograms of dicloxacillin per milligram.

(ii) It passes the safety test.

(iii) Its moisture content is not less than 3 percent nor more than 5 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 4.5 nor more than 7.5.

(v) Its organic chlorine content is not less than 13.0 percent nor more than 14.2 percent.

(vi) Its free chloride content is not more than 0.5 percent.

(vii) It is crystalline.

(viii) It gives a positive identity test for sodium dicloxacillin monohydrate.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, moisture, pH, organic chlorine content, free chloride content, crystallinity, and identity.

(ii) Samples required: 10 containers, each containing not less than 500 milligrams.

(b) *Tests and methods of assay—*(1) *Potency.* Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay, as follows: Dissolve an accurately weighed portion of the sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 5 micrograms of dicloxacillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter.

(iii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter.

(2) *Safety.* Proceed as directed in § 436.33 of this chapter.

(3) *Moisture content.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Organic chlorine content—*(i) *Reagents.* (*a*) *o*-Chlorobenzoic acid of known purity.

(*b*) 0.01*N* Silver nitrate solution. Store in brown glass reagent bottle. Standardize against an accurately weighed sample of 20 to 25 milligrams of *o*-chlorobenzoic acid using the procedure described in paragraph (b)(5)(ii) of this section.

$$\text{Normality } (N) = \frac{\text{Percent purity of the o-chlorobenzoic acid} \times \text{milligrams of o-chlorobenzoic acid}}{15{,}657 \times \text{milliliters of silver nitrate consumed}}$$

(*c*) 0.1*N* Sodium hydroxide solution.

(*d*) 1:1 Nitric acid solution: Mix 1 volume of concentrated nitric acid with 1 volume of distilled water.

(ii) *Total chlorine.* (Caution—The analyst should wear safety glasses and use a suitable shield between himself and the apparatus. The glassware must be scrupulously clean.) Accurately weigh 20 to 25 milligrams of the sample and place it on the center of a piece of halide-free filter paper measuring about 4 centimeters square (this is specially cut paper with a fuse strip attached to the area that holds the sample), and fold the paper to enclose it. Place 10 milliliters of 0.1*N* sodium hydroxide into an oxygen combustion flask (Schoniger flask), and flush the air from the flask with a stream of rapidly flowing oxygen. Place the sample into the platinum sample holder and ignite the fuse strip by suitable means. If the strip is ignited outside the flask, immediately plunge the stopper into the flask, invert so that the sodium hydroxide solution makes a seal around the stopper, and hold the stopper firmly in place. If the ignition is carried out in a closed system, the inversion of the flask may be omitted. After combustion is completed, shake the flask vigorously, add a small amount of distilled water to the collar to insure an air tight seal, and allow to stand for not less than 10 minutes with intermittent shaking Transfer to a suitable titration vessel, heat on a steam bath for 20 to 30 minutes, cool to room temperature, add 5 milliliters of nitric acid solution, and titrate potentiometrically with 0.01*N* silver nitrate, using one silver electrode and one silver/silver chloride electrode.

(iii) *Free chloride.* Accurately weigh 100 to 150 milligrams of sample directly into a titration flask, dissolve in 10 milliliters of 0.1*N* sodium hydroxide, and add about 20 milliliters of distilled water, Heat this solution on the steam bath 20 to 30 minutes. Cool to room temperature, add 5 milliliters of 1:1 nitric acid solution and titrate potentiometrically with 0.01*N* silver nitrate using one silver electrode and one silver/silver chloride electrode.

(iv) *Organic chlorine.* Percent organic chlorine=Percent total chlorine—percent free chloride.

(6) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(7) *Identity.* Proceed as directed in § 436.211 of this chapter, using the 1 percent potassium bromide disc described in paragraph (b)(1) of that section.

§ 440.19a Sterile sodium dicloxacillin monohydrate.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Sterile sodium dicloxacillin monohydrate is the monohydrated sodium salt of 5-methyl-3-(2,6-dichlorophenyl)-4-isoxazolyl penicillin. It is so purified and dried that:

(i) Its potency is not less than 850 micrograms of dicloxacillin per milligram. If it is packaged for dispensing, its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milli-

grams of dicloxacillin that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It passes the safety test.

(v) Its moisture content is not less than 3 percent and not more than 5 percent.

(vi) Its pH in an aqueous solution containing 10 milligrams per milliliter or when reconstituted as directed in the labeling, if it is packaged for dispensing is not less than 4.5 nor more than 7.5.

(vii) Its organic chlorine content is not less than 13.0 percent and not more than 14.2 percent.

(viii) Its free chloride content is not more than 0.5 percent.

(ix) It is crystalline.

(x) It gives a positive identity test for sodium dicloxacillin monohydrate.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, safety, moisture, pH, organic chlorine content, free chloride content, crystallinity, and identity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 15 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Sample preparation.* Dissolve an accurately weighed sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), for the microbiological agar diffusion assay and the hydroxylamine colorimetric assay or in distilled water for the iodometric assay, to give a stock solution of convenient concentration; and also if it is packaged for dispensing, reconstitute as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container, or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with either solution 1 or distilled water, as specified above, to give a stock solution of convenient concentration.

(ii) *Assay procedures.* Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 5 micrograms of dicloxacillin per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this subchapter.

(*c*) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this subchapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 20 milligrams of dicloxacillin per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this chapter.

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this subchapter, using an aqueous solution containing 10 milligrams per milliliter (or using a solution reconstituted as directed in the labeling if it is packaged for dispensing).

(7) *Organic chlorine content.* Proceed as directed in § 440.19(b)(5).

(8) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(9) *Identity.* Proceed as directed in § 436.211 of this chapter, using a 1 percent potassium bromide disc prepared as directed in paragraph (b)(1) of that section.

§ 440.25 Nonsterile hetacillin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Hetacillin is 6-(2,2-Dimethyl-5-oxo-4-phenyl-1-imidazolidinyl) - 3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid. It occurs as a fine, white to off-white powder. It is so purified and dried that:

(i) Its potency is not less than 810 micrograms of ampicillin per milligram.

(ii) It passes the safety test.

(iii) Its moisture content is not more than 1.0 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.5 nor more than 5.5.

(v) Its hetacillin content is not less than 90 and not more than 105 percent.

(vi) It gives a positive identity test for hetacillin.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, moisture, pH, hetacillin content, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed for ampicillin in § 436.105 of this chapter, using the ampicillin working standard as the standard of comparison and preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute the stock solution with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(2) *Safety.* Proceed as directed in § 436.33 of this chapter.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Hetacillin content*—(i) *Reagents*—(*a*) *Hydrochloric acid-acetone solution.* Dilute 8.5 milliliters of concentrated hydrochloric acid to 1 liter with acetone and mix well. Use for 1 day only.

(*b*) *p-Dimethylaminocinnamaldehyde solution.* Dissolve 0.5 gram of *p*-dimethylaminocinnamaldehyde in sufficient hydrochloric acid-acetone solution to a final volume of 100 milliliters and shake well, filtering if necessary. Prepare immediately before use.

(ii) *Preparation of standard solutions.* Transfer about 100 milligrams of the hetacillin working standard, accurately weighed, to a 200-milliliter volumetric flask. Add 150 milliliters of refrigerated distilled water and 20 milliliters of 1*N* hydrochloric acid, shake, dilute to volume with distilled water, and mix well. Transfer 0.5, 1.0, and 2.0 milliliters into three respective 25-milliliter volumetric flasks. Add 1.5 and 1.0 milliliters of 0.1*N* hydrochloric acid respectively to the first and second flasks to bring the volume in each to 2.0 milliliters.

(iii) *Blank.* Use 2.0 milliliters of 0.1*N* hydrochloric acid in a 25-milliliter volumetric flask.

(iv) *Preparation of sample solutions.* Using a mortar and pestle, grind the sample to a fine powder. Transfer an accurately weighed portion of about 100 milligrams to a 200-milliliter volumetric flask. Add 150 milliliters of refrigerated distilled water and 20 milliliters of 1*N* hydrochloric acid, shake, dilute to volume with distilled water, and mix well. Transfer 1.0 milliliter to a 25-milliliter volumetric flask, add 1.0 milliliter of 0.1*N* hydrochloric acid, and mix.

(v) *Procedure.* To each of the flasks containing standards, blank, and sample, add 15 milliliters of hydrochloric acid-acetone solution and mix. Then add 3 milliliters of *p*-dimethylaminocinnamaldehyde solution to each and mix. Add 3 milliliters of 0.1*N* hydrochloric acid to each, dilute to volume with hydrochloric acid-acetone solution, mix well, and allow to stand at 25° C. for exactly 30 minutes. (Filter the sample solutions, if necessary, to remove any turbidity.) Using a suitable spectrophotometer, read the absorbance values of standard and

sample solutions at a wavelength of 515 nanometers against the blank. Plot the absorbance values of the standards versus their concentrations and read the sample concentration from this standard response line.

(vi) *Calculations.*

$$\text{Percent hetacillin} = \frac{c \times 5{,}000 \times 100}{\text{Wt. of sample in milligrams}}$$

where:

c = Concentration in milligrams of hetacillin per milliliter of the final solution of the sample obtained from the standard response line.

5,000 = Dilution factor (200 × 25).

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using a 1 percent potassium bromide disc prepared as directed in paragraph (b)(1) of that section.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

§ 440.29 Nonsterile potassium hetacillin.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality and purity.* Potassium hetacillin is the potassium salt of hetacillin. It occurs as a fine, white to light buff powder. It is so purified and dried that:

(i) Its potency is not less than 735 micrograms of ampicillin per milligram.

(ii) It passes the safety test.

(iii) Its moisture content is not more than 1.0 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 7.0 and not more than 9.0.

(v) Its potassium hetacillin content is not less than 90 percent and not more than 105 percent.

(vi) It gives a positive identity test for potassium hetacillin.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, moisture, pH, potassium hetacillin content, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed for ampicillin in § 436.105 of this chapter, using the ampicillin working standard as the standard of comparison and preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute the stock solution with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(2) *Safety.* Proceed as directed in § 436.33 of this chapter.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Potassium hetacillin content.* Proceed as directed in § 440.25(b)(5), except use about 110 milligrams of sample and calculate the potassium hetacillin content as follows:

$$\text{Percent potassium hetacillin} = \frac{c \times 5{,}000 \times 427.57 \times 100}{\text{Wt. of sample in milligrams} \times 389.48}$$

where:

c = Concentration in milligrams of hetacillin per milliliter of the final solution of the sample obtained from the standard response line.

5,000 = Dilution factor (200 × 25).

427.57 = Molecular weight of potassium hetacillin.

389.48 = Molecular weight of hetacillin.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using a 1 percent potassium bromide disc prepared as directed in paragraph (b)(1) of that section.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

§ 440.29a Sterile potassium hetacillin.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Potassium hetacillin is the potassium salt of hetacillin. It occurs as a fine, white to light buff powder. It is so purified and dried that:

(i) Its potency is not less than 735 micrograms of ampicillin per milligram. If it is packaged for dispensing, its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of ampicillin that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It passes the safety test.

(v) Its moisture content is not more than 1.0 percent.

(vi) Its pH in an aqueous solution containing 10 milligrams per milliliter (or when reconstituted as directed in the labeling, if it is packaged for dispensing) is not less than 7.0 and not more than 9.0.

(vii) Its potassium hetacillin content is not less than 90 percent and not more than 105 percent.

(viii) It gives a positive identity test for potassium hetacillin.

(ix) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, safety, moisture, pH, potassium hetacillin content, identity, and crystallinity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 10 immediate containers, except if each contains less than 450 milligrams, a minimum of 16 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed for ampicillin in § 436.105 of this chapter, using the ampicillin working standard as the standard of comparison and preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration; and also if it is packaged for dispensing, reconstitute as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove the withdrawable contents from each container represented as a single-dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, withdraw an accurately measured representative portion from each container. Dilute the sample thus obtained with sufficient solution 3 to give a stock solution of convenient concentration. Further dilute the stock solution with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter using a solution containing the equivalent of 18 milligrams of ampicillin per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this chapter.

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter (or using a solution reconstituted as directed in the labeling, if it is packaged for dispensing).

(7) *Potassium hetacillin content.* Proceed as directed in § 440.25(b)(5), except use about 110 milligrams of sample and calculate the potassium hetacillin content as follows:

$$\text{Percent potassium hetacillin} = \frac{c \times 500 \times 427.57}{\text{Wt. of sample in milligrams} \times 389.48}$$

where:

c = Concentration in micrograms per milligram of the sample solution obtained from the standard response line.

(8) *Identity.* Proceed as directed in § 436.211 of this chapter, using a 1 percent potassium bromide disc prepared as directed in paragraph (b)(1) of that section.

(9) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

§ 440.36a Sterile sodium methicillin.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Sodium methicillin is the monohydrated sodium salt of (2,6-dimethoxyphenyl) penicillin. It is so purified and dried that:

(i) It contains not less than 815 micrograms of methicillin per milligram.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It passes the safety test.

(v) Its moisture content is not less than 3 percent and not more than 6 percent.

(vi) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 5.0 and not more than 7.5.

(vii) Its sodium methicillin content is not less than 90 percent.

(viii) It is crystalline.

(ix) It passes the identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, safety, mosture, pH, sodium methicillin content, crystallinity, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams, plus one package containing approximately 2 grams.

(*b*) For sterility testing: 20 packages, each containing approximately 600 milligrams.

(b) *Tests and methods of assay—*(1) *Potency.* Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 10 micrograms of methicillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this subchapter.

(iii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this subchapter.

(2) *Sterility.* Proceed as directed in in § 436.20 of this subchapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this subchapter, using a solution containing 20 milligrams of methicillin per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this subchapter.

(5) *Moisture.* Proceed as directed in § 436.201 of this subchapter.

(6) *pH.* Proceed as directed in § 436.202 of this subchapter, using an aqueous solution containing 10 milligrams per milliliter.

(7) *Sodium methicillin content.* Dissolve an accurately weighed portion of the sample in a sufficient accurately measured volume of distilled water to obtain a concentration of 0.2 milligram of sodium methicillin per milliliter (estimated). Treat a portion of the methicillin working standard in the same manner. Using a suitable spectrophotometer equipped with a 1-centimeter quartz cell and distilled water as the blank, determine the absorbance at 280 nanometers. If a recording spectrophotometer is used, record the ultraviolet absorption spectrum from 250 nanometers to 300 nanometers. If a nonrecording spectrophotometer is used, determine the absorbance (on a solution containing 10 milligrams per 100 milliliters) at the 280-nanometer absorption peak. (The exact position of the peak should be determined for the particular instrument used.) Calculate as follows:

$$\text{Percent sodium methicillin} = \frac{\text{Absorbance of sample} \times \text{Weight of working standard} \times \text{Volume of sample solution} \times \text{Percent sodium methicillin in working standard}}{\text{Absorbance of standard} \times \text{Weight of sample} \times \text{Volume of standard solution}}$$

(8) *Crystallinity.* Proceed as directed in § 436.203(a) of this subchapter.

(9) *Identity.* Using the sample solution prepared as described in paragraph (b)(7) of this section, determine the absorbancies at the absorption maximum at 280 nanometers and at the absorption minimum at 264 nanometers. The ratio of the two

$$\frac{A\,280}{A\,264}$$

should be not less than 1.30 and not more than 1.45.

§ 440.41 Nonsterile sodium nafcillin monohydrate.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Sodium nafcillin monohydrate is the monohydrated sodium salt of 6-(2-ethoxy-1-naphthamido) penicillanic acid. It is so purified and dried that:

(i) It contains not less than 820 micrograms of nafcillin per milligram.

(ii) It passes the safety test.

(iii) Its moisture content is not less than 3.5 percent and not more than 5.3 percent.

(iv) Its pH in an aqueous solution containing 30 milligrams per milliliter is not less than 5.0 and not more than 7.0.

(v) It is crystalline.

(vi) Its sodium nafcillin monohydrate content is not less than 90 percent.

(vii) It gives a positive identity test for nafcillin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 (b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, moisture, pH, crystallinity, sodium nafcillin monohydrate content, and identity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay—*(1) *Potency.* Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 2 micrograms of nafcillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter.

(iii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter.

(2) *Safety.* Proceed as directed in § 436.33 of this chapter.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 30 milligrams per milliliter.

(5) *Crystallinity.* Proceed as directed in § 436.203(b) of this chapter.

(6) *Sodium nafcillin monohydrate content.* Dissolve an accurately weighed portion of the sample in a sufficient accurately measured volume of distilled water to obtain a concentration of 0.05 milligram of sodium nafcillin monohydrate per milliliter (estimated). Treat a portion of the nafcillin working stand-

ard in the same manner. Using a suitable spectrophotometer equipped with quartz cells and distilled water as a blank, scan the absorption spectra of the sample and the nafcillin working standard solutions between the wavelengths of 245 nanometers and 340 nanometers. Determine the absorbance of the sample and working standard solutions at the absorption maximum at 280±3 nanometers. (The exact position of the maximum should be determined for the particular instrument used.) Calculate as follows:

$$\text{Percent sodium nafcillin monohydrate} = \frac{\text{Absorbance of sample} \times \text{weight in milligrams of standard} \times \text{volume of sample solution} \times \text{sodium nafcillin monohydrate content of standard in percent}}{\text{Absorbance of standard} \times \text{weight in milligrams of sample} \times \text{volume of standard solution}}$$

(7) *Identity.* The absorption spectrum of the sample determined as directed in paragraph (b)(6) of this section compares qualitatively with that of the nafcillin working standard.

§ 440.41a Sterile sodium nafcillin monohydrate.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Sterile sodium nafcillin monohydrate is the monohydrated sodium salt of 6-(2-ethoxy-1-naphthamido) penicillanic acid. It is so purified and dried that:

(i) It contains not less than 820 micrograms of nafcillin per milligram.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It passes the safety test.

(v) Its moisture content is not less than 3.5 nor more than 5.3 percent.

(vi) Its pH in an aqueous solution containing 30 milligrams per milliliter is not less than 5.0 and not more than 7.0.

(vii) It is crystalline.

(viii) Its sodium nafcillin monohydrate content is not less than 90 percent.

(ix) It gives a positive identity test for nafcillin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, safety, moisture, pH, crystallinity, sodium nafcillin monohydrate content, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay—*(1) *Potency.* Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 2 micrograms of nafcillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter.

(iii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 20 milligrams of nafcillin per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this chapter.

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 30 milligrams per milliliter.

(7) *Crystallinity.* Proceed as directed in § 436.203(b) of this chapter.

(8) *Sodium nafcillin monohydrate content.* Proceed as directed in § 440.41(b)(6).

(9) *Identity.* The absorption spectrum of the sample determined as directed in paragraph (b)(8) of this section compares qualitatively with that of the nafcillin working standard.

§ 440.49 Sodium oxacillin.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Sodium oxacillin is the monohydrated sodium salt of 5-methyl-3-phenyl-4-isoxazolyl penicillin. It is so purified and dried that:

(i) It contains not less than 815 and not more than 950 micrograms of oxacillin per milligram.

(ii) It passes the safety test.

(iii) Its moisture content is not less than 3.5 and not more than 5.0 percent.

(iv) Its pH in an aqueous solution containing 30 milligrams per milliliter is not less than 4.5 and not more than 7.5.

(v) Its sodium oxacillin content is not less than 90 percent and not more than 105 percent.

(vi) It is crystalline.

(vii) It gives a positive identity test for the oxacillin moiety.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, moisture, pH, sodium oxacillin content, crystallinity, and identity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay—*(1) *Potency.* Assay for potency by any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 5.0 micrograms of oxacillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter.

(iii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter.

(2) *Safety.* Proceed as directed in § 436.33 of this chapter.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution containing 30 milligrams per milliliter.

(5) *Sodium oxacillin content.* Place approximately 60 milligrams of sample, accurately weighed, into a 100-milliliter volumetric flask. Dissolve and fill to volume with distilled water. Pipette a 5.0-milliliter aliquot of the sample solution into a 22- by 200-milliliter test tube, and add 5 milliliters of 10*N* NaOH. Mix the solution, and place the tube in a boiling water bath for 60 minutes. Cool the tube, carefully add 10 milliliters of 6*N* HCL, mix, and replace the tube in the boiling water bath for 10 minutes. Position the tube in the bath so that the liquid level in the tube is the same as the liquid level in the bath. After heating, remove the tube from the bath, carefully agitate the contents of the tube, and cool to room temperature. Quantitatively transfer the contents of the tube to a 250-milliliter volumetric flask. Add approximately 200 milliliters of freshly boiled and cooled distilled water, then 4.0 milliliters of 7.5*N* NH_4OH, and dilute to volume with freshly boiled and cooled distilled water. Treat a sample of the oxacillin working standard in the same manner. Determine the absorbance of the sample and working standard solutions on a suitable spectrophotometer at 235 nanometers against a reagent blank, and calculate as follows:

$$\text{Percent sodium oxacillin} = \frac{\text{Absorbance of sample} \times \text{Weight in milligrams of standard} \times \text{Sodium oxacillin content of standard in percent}}{\text{Absorbance of standard} \times \text{Weight in milligrams of sample}}$$

(6) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(7) *Identity.* Use the sample solution prepared as described in paragraph (b)(5) of this section and record the ultraviolet spectrum between 230 nanometers and 260 nanometers. It should be basically identical to that of the standard similarly treated.

§ 440.49a Sterile sodium oxacillin.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Sterile sodium oxacillin is the monohydrated sodium salt of 5-methyl-3-phenyl-4-isoxazolyl penicillin. It is so purified and dried that:

(i) It contains not less than 815 and not more than 950 micrograms of oxacillin per milligram.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It passes the safety test.

(v) Its moisture content is not less than 3.5 and not more than 5.0 percent.

(vi) Its pH in an aqueous solution containing 30 milligrams per milliliter is not less than 4.5 and not more than 7.5.

(vii) Its sodium oxacillin content is not less than 90 percent and not more than 105 percent.

(viii) It is crystalline.

(ix) It gives a positive identity test for the oxacillin moiety.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, safety, moisture, pH, sodium oxacillin content, crystallinity, and identity.

(ii) Samples required:

(a) For all tests except sterility: 10 packages, each containing approximately 300 milligrams, plus one package containing approximately 2 grams.

(b) For sterility testing: 20 packages, each containing approximately 600 milligrams.

(b) *Tests and methods of assay—*(1) *Potency.* Assay for potency by any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 5.0 micrograms of oxacillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter.

(iii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 20 milligrams of oxacillin per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this chapter.

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution containing 30 milligrams per milliliter.

(7) *Sodium oxacillin content.* Proceed as directed in § 440.49(b)(5).

(8) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(9) *Identity.* Proceed as directed in § 440.49(b)(7).

§ 440.51 Penicillamine.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Penicillamine is D-3-mercaptovaline. It is so purified and dried that:

(i) Its content of penicillamine is not less than 97.0 percent and not more than 100.5 percent, calculated on a dried basis.

(ii) It passes the safety test.

(iii) Its loss on drying is not more than 0.5 percent.

(iv) Its pH in a 1 percent aqueous solution is not less than 4.5 and not more than 5.5.

(v) It contains not more than 0.2 unit of penicillin activity per gram.

(vi) Its residue on ignition is not more than 0.1 percent.

(vii) Its heavy metals content is not more than 20 parts per million.

(viii) Its specific rotation in a 1.0 *N* sodium hydroxide solution at 25° C. is −63°±5°.

(ix) It gives a positive identity test for penicillamine.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for penicillamine content, safety, loss on drying, pH, penicillin activity, residue on ignition, heavy metals, specific rotation, and identity.

(ii) Samples required: 11 packages, each containing 1 gram.

(b) *Tests and methods of assay—*(1) *Penicillamine content—*(i) *Reagents—*(a) *0.05 M mercuric acetate.* Dissolve 16 grams of mercuric acetate in water, add 5 milliliters of glacial acetic acid, and dilute to 1,000.0 milliliters with water.

(b) *Diphenylcarbazone solution.* Dissolve 0.5 gram of diphenylcarbazone in 100 milliliters of 95 percent alcohol.

(ii) *Standardization of 0.05 mercuric acetate solution.* Dissolve approximately 300 milligrams of the penicillamine working standard, accurately weighed, in 200 milliliters of water. Add 10 grams of sodium acetate, acidify the solution to litmus paper with acetic acid, add 1.0 milliliter of a freshly prepared solution of diphenylcarbazone, and titrate with mercuric acetate until a rose-violet color persists for 2 to 3 minutes. Determine the penicillamine equivalence in milligrams for each milliliter of 0.05 *M* mercuric acetate by means of the following calculation:

$$A = \frac{W_1 \times P}{V_1 \times 100}$$

where:

A = Penicillamine equivalence in milligrams per milliliter of the mercuric acetate reagent.

V_1 = Total volume in milliliters of 0.05 *M* mercuric acetate used to titrate the penicillamine working standard.

W_1 = Weight of the penicillamine working standard in milligrams.

P = Purity of the penicillamine working standard in percent.

(iii) *Procedure.* Proceed as directed in paragraph (b)(1)(ii) of this section, except use approximately 300 milligrams of the sample and calculate the penicillamine content by means of the following formula:

$$\text{Penicillamine content in percent} = \frac{V_2 \times A \times 100}{W_2}$$

where:

V_2 = Total volume in milliliters of mercuric acetate reagent used to titrate the sample;

W_2 = Weight of the sample in milligrams.

(2) *Safety.* Proceed as directed in § 436.33 of this chapter, preparing the test dose solution as follows: Grind the sample in a mortar to a fine powder. With continued grinding, suspend the powder in sufficient diluent 10 (as listed in § 436.31) to give the prescribed concentration, adding 1 drop of U.S.P. polysorbate 80 for each gram of penicillamine.

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a 1 percent aqueous solution.

(5) *Penicillin activity.* Proceed as directed in § 436.104 of this chapter.

(6) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(7) *Heavy metals content.* Proceed as directed in § 436.208 of this chapter.

(8) *Specific rotation.* Proceed as directed in § 436.210 of this chapter, using a 5.0 percent (weight per volume) solution of penicillamine in 1.0*N* sodium hydroxide and a tube 2.0 decimeters in length. Calculate the specific rotation on an anhydrous basis.

(9) *Identity.* Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b)(2) of that section, except use exactly 50 milligrams of sample and 300 milligrams of mineral oil.

§ 440.53a Sterile aluminum penicillin (aluminum penicillin salt).

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Aluminum penicillin is the insoluble aluminum salt of a kind of penicillin or a mixture of two or more such salts, but the quantity of any salt of penicillin K therein is not more than 30 percent. Each such drug is so purified and dried that:

(i) Its potency is not less than 500 units per milligram;

(ii) It is sterile;

(iii) It is nonpyrogenic;

(iv) It is nontoxic;

(v) Its moisture content is not more than 3 percent; and

(vi) Its pH in saturated aqueous solution is not less than 3.5 and not more than 4.5.

(2) *Packaging.* In all cases the immediate containers shall be tight containers as defined by the U.S.P., shall be sterile at the time of filling and closing, shall be so sealed that the contents cannot be used without destroying the seal, and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* Each package of aluminum penicillin shall bear on its outside wrapper or container and the immediate container as hereinafter indicated, the following:

(i) The batch mark;

(ii) The weight of the drug and the number of units in the immediate container;

(iii) The statement "Expiration date ---------", the blank being filled in with the date which is 12 months after the month during which the batch was certified;

(iv) The statement "Store in refrigerator not above 15° C. (59° F.)" or "Store below 15° C. (59° F.)";

(v) The statement "For manufacturing use only";

(vi) The statement "Caution: Federal law prohibits dispensing without prescription".

(4) *Request for certification, check tests and assays; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch of aluminum penicillin shall submit with his request a statement showing the batch mark, the number of packages of each size in the batch, the weight of the drug and the number of units in each package, and (unless it was previously submitted) the date on which the latest assay of the drug comprising the batch was completed. Such request shall be accompanied or followed with results of the tests and assays made by him on the batch for potency, sterility, pyrogens, toxicity, moisture, pH, and the penicillin K content.

(ii) Such person shall submit in connection with his request an accurately representative sample of the batch, consisting of the following:

(*a*) For all tests except sterility: Six packages, containing approximately equal portions of not less than 60 milligrams each.

(*b*) For sterility testing: 20 packages each containing approximately 600 milligrams.

Each such portion shall be taken from a different part of such batch and each shall be packaged in accordance with the requirements of paragraph (a) (2) of this section.

(iii) In connection with contemplated requests for certification of batches of another drug in the manufacture of which aluminum penicillin is to be used, the manufacturer of a batch which is to be so used may request the Commissioner to make check tests and assays on a sample of such batch taken as prescribed by paragraph (a) (4) (ii) of this section. From the information required by paragraph (a) (4) (i) of this section may be omitted results of tests and assays not required for the batch when used in such other drug. The Commissioner shall report to such manufacturer results of each check test and assay as are so requested.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 440.80a(b) (1), using citrate buffer as prepared in § 440.153(b) (1) in lieu of phosphate buffer.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e) (2) of that section, except use medium B in lieu of medium A.

(3) *Pyrogens.* Proceed as directed in § 440.80a(b) (3), but in lieu of the directions for preparation of sample in paragraph (b) (3) (ii) thereof, prepare sample as follows:

Suspend approximately 60 milligrams in 20 milliliters of pyrogen-free sterile physiological salt solution, adding the salt solution in approximately 1-milliliter aliquots and mixing thoroughly after each addition, utilizing a pyrogen-free glass stirring rod. Centrifuge, warm to 37° C., withdraw the clear supernatant solution, and inject 1 milliliter per kilogram of rabbit.

(4) *Toxicity.* Proceed as directed in § 440.80a(b) (4), utilizing the solution prepared in paragraph (b) (2) of this section.

(5) *Moisture.* Proceed as directed in § 440.80a(b) (5) (i).

(6) *pH.* Proceed as directed in § 440.80a(b) (5) (ii), using a saturated solution.

(7) *Penicillin K content.* Proceed as directed in § 440.80a(b) (5) (vi).

§ 440.55a Sterile benzathine penicillin G (benzathine penicillin G salt).

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Benzathine penicillin G is the crystalline *N,N'*-dibenzylethylenediamine salt of penicillin G. It contains not less than 85 percent by weight of the *N,N'*-dibenzylethylenediamine salt of penicillin G. Each such drug is so purified and dried that:

(i) Its potency is not less than 1050 units per milligram.

(ii) It is sterile.

(iii) It is nontoxic.

(iv) It is nonpyrogenic.

(v) Its moisture content is not more than 8 percent.

(vi) Its pH in a saturated aqueous solution is not less than 5.0 and not more than 7.5.

(2) *Packaging.* In all cases the immediate containers shall be tight containers as defined by the U.S.P., shall be sterile at the time of filling and closing, shall be so sealed that the contents cannot be used without destroying the seal, and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* Each package shall bear on its outside wrapper or container and the immediate container, as hereinafter indicated, the following:

(i) The batch mark.

(ii) The weight of the drug and the number of units in the immediate container.

(iii) The statement "Expiration date ---------", the blank being filled in with the date which is 36 months after the month during which the batch was certified.

(iv) The statement "For manufacturing use only".

(v) The statement "Caution: Federal law prohibits dispensing without prescription".

(4) *Request for certification, check tests and assays; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in the batch, the weight of the drug and the number of units in each package, and (unless it was previously submitted) the date on which the latest assay of the drug comprising such batch was completed. Such request shall be accompanied or followed by the results of tests and assays made by him on the batch for potency, sterility, toxicity, pyrogens, moisture, pH, crystallinity, and the penicillin G content.

(ii) Such person shall submit with his request an accurately representative sample of the batch, consisting of the following:

(*a*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 600 milligrams.

Each such package shall be taken from a different part of such batch and each shall be packaged in accordance with the requirements of paragraph (a) (2) of this section.

(iii) In connection with contemplated requests for certification of batches of another drug in the manufacture of

which benzathine penicillin G is to be used, the manufacturer of a batch which is to be so used may request the Commissioner to make check tests and assays on a sample of such batch, taken as prescribed by paragraph (a)(4)(ii) of this section. From the information required by paragraph (a)(4)(i) of this section may be omitted results of tests and assays not required for the batch when used in such other drug. The Commissioner shall report to such manufacturer results of such check tests and assays as are so requested.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 440.80a(b)(1), except if the bioassay method is used dissolve the sample to be tested in sufficient dimethyl formamide, formamide or redistilled methanol prior to diluting with phosphate buffer solution. If the iodometric method is used proceed as directed in § 440.80a(b)(5)(iv), except in preparing the blank solution weigh 30–60 milligrams of the sample and make a suspension of 2 milligrams per milliliter in 1-percent phosphate buffer at pH 6.0. Shake well, pipette 2.0 milliliters into a 125-milliliter glass-stoppered Erlenmeyer flask, add 10 milliliters 0.01*N* iodine and immediately titrate with 0.01*N* $Na_2S_2O_3$. In preparing the solution for inactivation dissolve a weighed sample (30–60 milligrams) in sufficient 1*N* NaOH to give 2.0 milligrams per milliliter. Pipette a 2.0-milliliter aliquot into a 125-milliliter glass-stoppered Erlenmeyer flask, and after 15 minutes add 2.0 milliliters 1.2 *N* HCl and 10 milliliters 0.01*N* iodine. Allow to stand for 15 minutes and titrate the excess iodine with 0.01*N* $Na_2S_2O_3$.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except use medium C in lieu of medium A, medium F in lieu of medium E, and during the period of incubation shake the tubes at least once daily.

(3) *Toxicity.* Proceed as directed in § 440.80a(b)(4), except use physiological salt solution as the diluent and inject 0.25 milliliter of a suspension containing 4,000 units per milliliter.

(4) *Pyrogens.* Proceed as directed in § 440.80a(b)(3), except use physiological salt solution as the diluent and inject 0.5 milliliter per kilogram of rabbit of a suspension containing 4,000 units per milliliter.

(5) *Moisture.* Proceed as directed in § 440.74a(b)(5).

(6) *pH. Proceed as directed in* § 440.80a(b)(5)(ii), using a saturate aqueous solution prepared by adding 5 milligrams per milliliter.

(7) *Microscopical test for crystallinity.* Proceed as directed in § 440.80a(b)(5)(iii).

(8) *Penicillin G content.* Dissolve 50 milligrams of the sample, accurately weighed, in absolute methyl alcohol and make to a volume of 100 milliliters with absolute methyl alcohol. Using a suitable spectrophotometer, determine the absorbance of the solution in a 1-centimeter cell at 263 mμ compared with absolute methyl alcohol as a blank.

$$\text{Percent benzathine penicillin G} = \frac{\text{Absorbance at 263 m}\mu \times 100{,}000}{\text{Milligrams of sample (per 100 milliliters)} \times a_{263}},$$

where:

a_{263} = absorptivity (1%, 1 cm.) of benzathine penicillin G working standard, similarly treated.

§ 440.57a Sterile benzathine phenoxymethyl penicillin (benzathine phenoxymethyl penicillin salt).

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Benzathine phenoxymethyl penicillin is the crystalline benzathine salt of phenoxymethyl penicillin. It contains not less than 90 percent by weight of the benzathine salt of phenoxymethyl penicillin. It is so purified and dried that:

(i) Its potency is not less than 1,050 units per milligram.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It is nontoxic.

(v) Its moisture content is not more than 8.0 percent.

(vi) Its pH in a saturated aqueous solution is not less than 4.0 and not more than 6.5.

(2) *Packaging.* In all cases the immediate container shall be a tight container as defined by the U. S. P. and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused that are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* Each package shall bear on its outside wrapper or container and the immediate container, as hereinafter indicated, the following:

(i) The batch mark.

(ii) The number of units per milligram and the number of grams in the immediate container.

(iii) The statement "Expiration date ----------", the blank being filled in with the date that is 24 months after the month during which the batch was certified.

(iv) The statement "For manufacturing use only".

(v) The statement "Caution: Federal law prohibits dispensing without prescription".

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in the batch, and the date on which the latest assay of the drug comprising such batch was completed. Such request shall be accompanied or followed by the results of tests and assays made by him on the batch for potency, sterility, pyrogens, toxicity, moisture, pH, crystallinity, and phenoxymethyl penicillin content.

(ii) Each person shall submit with his request an accurately representative sample of the batch, consisting of the following:

(*a*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 600 milligrams.

Each such package shall be taken from a different part of such batch, and each shall be packaged in accordance with the requirements of paragraph (a)(2) of this section.

(iii) In connection with contemplated requests for certification of batches of another drug in the manufacture of which benzathine phenoxymethyl penicillin is to be used, the manufacturer of a batch that is to be so used may request the Commissioner to make check tests and assays on a sample of such batch, taken as prescribed in paragraph (a)(4)(ii) of this section. From the information required by paragraph (a)(4)(i) of this section may be omitted results of tests and assays not required for the batch when used in such other drug. The Commissioner shall report to such manufacturer results of such check tests and assays as are so requested.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 440.55a(b)(1), except use the phenoxymethyl penicillin working standard as the standard of comparison.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except use medium C in lieu of medium A, and medium F in lieu of medium E. During the period of incubation, shake the tubes at least once daily.

(3) *Pyrogens.* Proceed as directed in § 440.80a(b)(3), except use sodium chloride solution as the diluent and inject 0.5 milliliter per kilogram of rabbit of a suspension containing 4,000 units per milliliter.

(4) *Toxicity.* Proceed as directed in § 440.80a(b)(4), except use sodium chloride solution as the diluent and inject 0.25 milliliter of a suspension containing 4,000 units per milliliter.

(5) *Moisture.* Proceed as directed in § 440.74a(b)(5).

(6) *pH.* Proceed as directed in § 440.80a(b)(5)(ii), using a saturated aqueous solution prepared by adding 5 milligrams per milliliter.

(7) *Microscopical test for crystallinity.* Proceed as directed in § 440.80a(b)(5)(iii).

(8) *Phenoxymethyl penicillin content.* Using the spectrophotometric method, proceed as directed in § 440.71(b)(6), except that the calculations are as follows:

$$\text{Percent benzathine phenoxymethyl penicillin} = \frac{\text{Absorbance at 276 m}\mu \times 100{,}000 \times 100}{\text{Milligrams of sample (in 100 milliliters)} - a_w \times 69.2}$$

where:

a_w = absorptivity (1%, 1 cm.) of the phenoxymethyl penicillin working standard similarly treated.

69.2 = percentage of phenoxymethyl penicillin in pure benzathine phenoxymethyl penicillin.

§ 440.59a Sterile chloroprocaine penicillin O (penicillin O chloroprocaine salt).

(a) *Requirements for certification—*

(1) *Standards of identity, strength, quality, and purity.* Chloroprocaine penicillin O is the crystalline 2-chloroprocaine salt of penicillin O prepared from 2-chloroprocaine hydrochloride (95 percent purity and a melting point of 171°–176° C.) and crystalline penicillin O. It contains not less than 85 percent of the 2-chloroprocaine salt of penicillin O and not more than 0.5 percent of the 2-chloroprocaine salt of penicillin G. Each such drug is so purified and dried that:

(i) Its potency is not less than 850 units per milligram.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It is nontoxic.

(v) Its moisture content is not more than 4.2 percent.

(vi) Its pH in saturated aqueous solution is not less than 5.0 and not more than 7.5.

(2) *Packaging.* In all cases the immediate containers shall be tight containers as defined by the U.S.P., shall be sterile at the time of filling and closing, shall be so sealed that the contents cannot be used without destroying the seal, and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribtuion practice shall be disregarded.

(3) *Labeling. Each package of* chloroprocaine penicillin O shall bear on its outside wrapper or container and the immediate container, as hereinafter indicated, the following:

(i) The batch mark.

(ii) The weight of the drug and the number of units in the immediate container.

(iii) The statement "Expiration date ----------", the blank being filled in with the date which is 36 months after the month during which the batch was certified.

(iv) The statement "For manufacturing use only".

(v) The statement "Caution: Federal law prohibits dispensing without prescription".

(4) *Request for certification, check tests and assays; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch of chloroprocaine penicillin O shall submit with his request a statement showing the batch mark, the number of packages of each size in the batch, the weight of the drug and the number of units in each package, and (unless it was previously submitted) the date on which the latest assay of the drug comprising the batch was completed. Such request shall be accompanied or followed by the results of tests and assays made by him on the batch for potency, sterility, toxicity, pyrogens, moisture, pH, crystallinity, chloroprocaine penicillin O content, and chloroprocaine penicillin G content.

(ii) Such person shall submit in connection with his request an accurately representative sample of the batch:

(*a*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 600 milligrams.

Each such package shall be taken from a different part of such batch, and each shall be packaged in accordance with the requirements of paragraph (a)(2) of this section.

(iii) In connection with contemplated requests for certification of batches of another drug in the manufacture of which chloroprocaine penicillin O is to be used, the manufacturer of a batch which is so used may request the Commissioner to make check tests and assays on a sample of such batch taken as prescribed by paragraph (a)(4)(ii) of this section. From the information required by paragraph (a)(4)(i) of this section may be omitted results of tests and assays not required for the batch when used in such other drug. The Commissioner shall report to such manufacturer results of such check tests and assays as are so requested.

(b) *Tests and methods of assay—*

(1) *Potency, sterility, pyrogens, toxicity, moisture, pH, and crystallinity.* Proceed as directed in § 440.74a(b), except if the iodometric assay for potency is used, calculate as directed in § 440.80a(b)(5)(iv)(*b*).

(2) *Chloroprocaine penicillin O content.* Proceed as directed in § 440.80a(b)(5)(viii)(*a*), except use an accurately weighed sample of approximately 168 milligrams and calculate the percent of chloroprocaine penicillin O as follows:

$$\frac{a(\text{sample})}{a(\text{standard})} \times 168 = \text{percent chloroprocaine penicillin O.}$$

(3) *Chloroprocaine penicillin G content.* Proceed as directed in § 440.80a(b)(5)(vii), except prepare the sample as follows: Accurately weigh approximately 500 milligrams of the sample in a 25-milliliter glass-stoppered test tube. Add 6 milliliters of distilled water and 6.0 milliliters of amyl acetate. Place the tube in an ice bath for 5 minutes and then add 4 milliliters of 1–4 H_3PO_4, shake vigorously for 2 minutes, centrifuge for 1 minute to separate the layers, and withdraw 5.0 milliliters of the amyl acetate layer. Place the amyl acetate in a 250-milliliter Erlenmeyer flask and evaporate to dryness by directing a stream of air into the flask. Dissolve the residue in 2.0 milliliters of 2.5 percent KOH. Calculate the percent of penicillin G in the sample from the equation:

$$\frac{(5.10)(x)(50)(100)}{(\text{Wt. sample milligrams})(0.833)} = \text{percent penicillin G}$$

§ 440.60a Sterile dibenzylamine penicillin G (dibenzylamine penicillin G salt).

(a) *Requirement for certification—*

(1) *Standards of identity, strength, quality, and purity.* Dibenzylamine penicillin G is the crystalline dibenzylamine salt of penicillin G. It contains not less than 85 percent by weight of the dibenzylamine salt of penicillin G. Each such drug is so purified and dried that:

(i) Its potency is not less than 975 units per milligram.

(ii) It is sterile.

(iii) It is nontoxic.

(iv) It is nonpyrogenic.

(v) Its moisture content is not more than 4.0 percent.

(vi) Its pH in saturated aqueous solution is not less than 5.0 and not more than 7.5.

(2) *Packaging.* In all cases the immediate containers shall be tight containers as defined by the U. S. P., shall be sterile at the time of filling and closing, shall be so sealed that the contents cannot be used without destroying the seal, and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused that are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* Each package shall bear on its outside wrapper or container and the immediate container, as hereinafter indicated, the following:

(i) The batch mark.

(ii) The weight of the drug and the number of units in the immediate container.

(iii) The statement "Expiration date ----------", the blank being filled in with the date which is 18 months after the month during which the batch was certified.

(iv) The statement "For manufacturing use only".

(v) The statement "Caution: Federal law prohibits dispensing without prescription".

(4) *Request for certification, check tests and assays; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in the batch, the weight of the drug and the number of units in each package, and (unless it was previously submitted) the date on which the latest assay of the drug comprising

such batch was completed. Such request shall be accompanied or followed by the results of tests and assays made by him on the batch for potency, sterility, toxicity, pyrogens, moisture, pH, crystallinity, and the penicillin G content.

(ii) Such person shall submit with his request an accurately representative sample of the batch, consisting of the following:

(*a*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 600 milligrams.

Each such package shall be taken from a different part of such batch, and each shall be packaged in accordance with the requirements of paragraph (a)(2) of this section.

(iii) In connection with contemplated requests for certification of batches of another drug in the manufacture of which dibenzylamine penicillin G is to be used, the manufacturer of a batch that is to be so used may request the Commissioner to make check tests and assays on a sample of such batch, taken as prescribed by paragraph (a)(4)(ii) of this section. From the information required by paragraph (a)(4)(i) of this section may be omitted results of tests and assays not required for the batch when used in such other drug. The Commissioner shall report to such manufacturer results of such check tests and assays as are so requested.

(b) *Tests and methods of assay*—

(1) *Potency.* Proceed as directed in § 440.74a(b)(1).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except use medium B in lieu of medium A.

(3) *Pyrogens.* Proceed as directed in § 440.80a(b)(3), except use physiological salt solution as the diluent.

(4) *Toxicity.* Proceed as directed in § 440.80a(b)(4), except use physiological salt solution as the diluent, and inject 0.25 milliliter of a solution containing 4,000 units per milliliter.

(5) *Moisture.* Proceed as directed in § 440.74a(b)(5).

(6) *pH.* Proceed as directed in § 440.80a(b)(5)(ii), using a saturated aqueous solution prepared by adding 300 milligrams per milliliter.

(7) *Microscopical test for crystallinity.* Proceed as directed in § 440.80a(b)(5)(iii).

(8) *Penicillin G content.* Proceed as directed in § 440.74a(b)(8), using the following formula for calculating the percent of dibenzylamine penicillin G:

$$\text{Percent of dibenzylamine penicillin G} = \frac{\text{N-ethyl piperidine penicillin precipitates} \times 245.5}{\text{Weight of sample in milligrams}}$$

§ 440.61a Sterile diethylaminoethyl ester penicillin G hydriodide (penicillin G diethylaminoethyl ester hydriodide).

(a) *Requirements for certification*—

(1) *Standards of identity, strength, quality, and purity.* Diethylaminoethyl ester penicillin G hydriodide is the crystalline diethylaminoethyl ester hydriodide salt of penicillin G. It contains not less than 85 percent by weight of β-diethylaminoethyl ester hydriodide salt of penicillin G. It is so purified and dried that:

(i) Its potency after hydrolysis is not less than 900 units per milligram.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It is nontoxic.

(v) Its moisture content is not more than 1 percent.

(vi) The pH of a saturated aqueous solution is not less than 4.0 and not more than 6.5.

(2) *Packaging.* In all cases the immediate container shall be a tight container as defined by the U.S.P., shall be sterile at the time of filling and closing, shall be so sealed that the contents cannot be used without destroying the seal, and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* Each package shall bear, on its outside wrapper or container and the immediate container as hereinafter indicated, the following:

(i) The batch mark.

(ii) The weight of the drug and the number of units in the immediate container.

(iii) The statement "Expiration date ----------", the blank being filled in with the date which is 24 months after the month during which the batch was certified.

(iv) The statement "For manufacturing use only".

(v) The statement "Caution: Federal law prohibits dispensing without prescription".

(4) *Request for certification, check tests and assays; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in the batch, the weight of the drug and the number of units in each package, and (unless it was previously submitted) the date on which the latest assay of the drug comprising such batch was completed. Such request shall be accompanied or followed by the results of tests and assays made by him for potency, sterility, toxicity, pyrogens, moisture, pH, crystallinity, and the penicillin G content.

(ii) Such person shall submit with his request an accurately representative sample of the batch, consisting of the following:

(*a*) For all tests except sterility; 10 packages, each containing approximately 300 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 600 milligrams.

Each such package shall be taken from a different part of such batch, and each shall be packaged in accordance with the requirements of paragraph (a)(2) of this section.

(iii) In connection with contemplated requests for certification of batches of another drug in the manufacture of which it is to be used, the manufacturer of a batch which is to be so used may request the Commissioner to make check tests and assays on a sample of such batch, taken as prescribed by paragraph (a)(4)(ii) of this section. From the information required by paragraph (a)(4)(i) of this section may be omitted results of tests and assays not required for the batch when used in such other drugs. The Commissioner shall report to such manufacturer results of such check tests and assays as are so requested.

(b) *Tests and methods of assay*—

(1) *Potency.* Proceed as directed in § 440.80a(b)(1), except prepare the sample as follows: Dissolve in 0.1*M* potassium phosphate buffer (pH 7.8–8.0) to make a stock solution of 100 units per milliliter (estimated). Allow to stand at room temperature for not less than 1.5 hours and not more than 2 hours and then dilute an aliquot with 1-percent phosphate buffer (pH 6.0) to 1.0 unit (estimated), or proceed by the iodometric method described in § 440.80a(b)(5)(iv)(*a*), except prepare the sample as follows: Dissolve a weighed sample (approximately 50 milligrams) in 2.0 milliliters of redistilled methanol. Further dilute this solution with 1-percent phosphate buffer (pH 6.0) to give a concentration of 2,000 units per milliliter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except add sufficient penicillinase either to diluting fluid A, or, if the product contains lecithin, to diluting fluid D, and swirl the flask to completely solubilize the preparation.

(3) *Pyrogens.* Proceed as directed in § 440.80a(b)(3), except use physiological salt solution as the diluent.

(4) *Toxicity.* Proceed as directed in § 440.80a(b)(4), except use physiological salt solution as the diluent, and inject 0.5 milliliter of a solution containing 2,000 units per milliliter.

(5) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i).

(6) *pH.* Proceed as directed in § 440.80a(b)(5)(ii), using a saturated aqueous solution prepared by adding 300 milligrams per milliliter.

(7) *Microscopical test for crystallinity.* Proceed as directed in § 440.80a(b)(5)(iii).

(8) *Penicillin G content.* Accurately weigh approximately 50 milligrams of the sample and dissolve in 25-milliliter potassium phosphate buffer, pH 7.9 (one part of 0.1*M* KH_2PO_4 and 11.66 parts of 0.1*M* K_2HPO_4). Allow to stand at room temperature for not less than 1.5 hours and not more than 2 hours. Transfer a 5.0-milliliter aliquot to a 25-milliliter glass-stoppered test tube. Place the tube in an ice bath and add 10.0 milliliters of chloroform (previously washed with water). After cooling, adjust the pH of the aqueous phase to 2.0 by adding 0.5 milliliter of 1–4 H_3PO_4. Shake the tube thoroughly for 2 minutes, centrifuge, and withdraw the lower choroform layer with the aid of a 10-milliliter hypodermic syringe and a 3-inch needle. Superficially dry the chloroform by filtering through a pledget of cotton, using a U-shaped funnel to reduce evaporation during filtration. Determine the absorbance of the filtered chloroform solution in a 1-centimeter cell at 265 mμ and 280 mμ, using a suitable spectrophotometer. Treat the working standard of sodium penicillin G in the same manner, using an accurately weighed sample of approximately 30 milligrams.

$$\text{Percent penicillin G} = \frac{(A_u \text{ at } 265\ m\mu - A_u \text{ at } 280\ m\mu)\ (\text{wt. } s)\ (100)}{(A_s \text{ at } 265\ m\mu - A_s \text{ at } 280\ m\mu)\ (\text{wt. } x)\ (0.625)}$$

where:

A = absorbance.

x = diethylaminoethyl ester penicillin G hydriodide.

s = sodium penicillin G working standard.

§ 440.63a Sterile ephedrine penicillin (penicillin ephedrine salt), ephedrine penicillin G (penicillin G ephedrine salt).

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Ephedrine penicillin is the crystalline ephedrine salt of a kind of penicillin, or a mixture of two or more such salts prepared from ephedrine U. S. P. and penicillin, but the quantity of any salt of penicillin K therein is not more than 30 percent; ephedrine penicillin G is ephedrine penicillin which contains not less than 85 percent by weight of the ephedrine salt of penicillin G. Each such drug is so purified and dried that:

(i) Its potency is not less than 1,000 units per milligram;

(ii) It is sterile;

(iii) It is nontoxic;

(iv) It is nonpyrogenic;

(v) Its moisture content is not more than 1.5 percent;

(vi) Its pH aqueous solution of 30 milligrams per milliliter is not less than 5 and not more than 7.5.

(2) *Packaging.* In all cases the immediate containers shall be tight containers as defined by the U.S.P., shall be sterile at the time of filling and closing, shall be so sealed that the contents cannot be used without destroying the seal, and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* Each package of ephedrine penicillin shall bear on its outside wrapper or container and the immediate container as hereinafter indicated, the following:

(i) The batch mark;

(ii) The weight of the drug and the number of units in the immediate container;

(iii) The statement "Expiration date _____", the blank being filled in with the date which is 12 months after the month during which the batch was certified.

(iv) The statement "For manufacturing use only";

(v) The statement "Caution: Federal law prohibits dispensing without prescription".

(4) *Requests for certification, check tests and assays; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch of ephedrine penicillin shall submit with his request a statement showing the batch mark, the number of packages of each size in the batch, the weight of the drug and the number of units in each package, and (unless it was previously submitted) the date on which the latest assay of the drug comprising the batch was completed. Such request shall be accompanied or followed by the results of tests and assays made by him on the batch for potency, sterility, toxicity, pyrogens, moisture, pH, crystallinity, penicillin K content (unless it is ephedrine penicillin G), and the penicillin G content if it is ephedrine penicillin G.

(ii) Such person shall submit with his request an accurately representative sample of the batch, consisting of the following:

(*a*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 600 milligrams.

Each such package shall be taken from a different part of such batch, and each shall be packaged in accordance with the requirements of paragraph (a)(2) of this section.

(iii) In connection with the contemplated requests for certification of batches of another drug in the manufacture of which ephedrine penicillin is to be used, the manufacturer of a batch which is to be so used may request the Commissioner to make check tests and assays on a sample of such batch taken as prescribed by paragraph (a)(4)(ii) of this section. From the information required by paragraph (a)(4)(i) of this section may be omitted results of tests and assays not required for the batch when used in such other drug. The Commissioner shall report to each manufacturer results of such check tests and assays as are so requested.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 440.80a(b)(1), or by the iodometric method as described in § 440.80a(b)(5)(iv)(*a*), except dilute the sample with sufficient 1 percent phosphate buffer pH 6.0 to give a concentration of 2.0 milligrams per milliliter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) or (2) of that section, except if using the method in paragraph (e)(2), use medium B in lieu of medium A.

(3) *Pyrogens.* Proceed as directed in § 440.80a(b)(3).

(4) *Toxicity.* Proceed as directed in § 440.80a(b)(4).

(5) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i).

(6) *pH.* Proceed as directed in § 440.80a(b)(5)(ii).

(7) *Microscopical test for crystallinity.* Proceed as directed in § 440.80a(b)(5)(iii).

(8) *Penicillin G content.* Proceed as directed in § 440.80a(b)(5)(v) using an accurately weighed sample of approximately 80 mg. and the following formula for calculating the percent of ephedrine penicillin G:

$$\text{Percent of ephedrine penicillin G} = \frac{\text{Milligrams } N\text{-ethyl piperidine penicillin precipitate} \times 223.2}{\text{Weight of sample in milligrams}}$$

(9) *Penicillin K content.* Proceed as directed in § 440.80a(b)(5)(vi).

§ 440.65a Sterile *l*-ephenamine penicillin G (penicillin G *l*-ephenamine salt).

(a) *Requirements for certification—*

(1) *Standards of identity, strength, quality and purity.* *l*-Ephenamine penicillin G is the heat stable crystalline *levo*-N-methyl-1, 2-diphenyl-2-hydroxyethylamine salt of penicillin G, prepared from crystalline penicillin G and crystalline *dl*-N-methyl-1, 2-diphenyl-2-hydroxyethylamine hydrochloride (98 percent purity and a melting point of 268.5° C. ±2.5° C.). It contains not less than 85 percent by weight of the *levo*-N-methyl-1, 2-diphenyl-2-hydroxyethylamine salt of penicillin G. It is so purified and dried that:

(i) Its potency is not less than 900 units per milligram;

(ii) It is sterile;

(iii) It is nonpyrogenic;

(iv) It is nontoxic;

(v) Its moisture content is not more than 1.5 percent;

(vi) Its pH in a saturated aqueous solution is not less than 5 and not more than 7.5; and

(vii) Its specific rotation in water-acetone (1+1) at 20° C. is +125° ±5°.

(2) *Packaging.* In all cases the immediate container shall be a tight container as defined by the U. S. P., shall be sterile at the time of filling and closing, shall be so sealed that the contents cannot be used without destroying the seal, and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* Each package shall bear on its outside wrapper or container and the immediate container as hereinafter indicated, the following:

(i) The batch mark;

(ii) The weight of the drug and the number of units in the immediate container;

(iii) The statement "Expiration date ----------", the blank being filled in with the date which is 48 months after the month during which the batch was certified;

(iv) The statement "For manufacturing use only";

(v) The statement "Caution: Federal law prohibits dispensing without prescription".

(4) *Request for certification, check tests and assays; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in the batch, the weight of the drug and the number of units in each package, and (unless it was previously submitted) the date on which the latest assay of the drug comprising such batch was completed. Such request shall be accompanied or followed by the results of tests and assays made by him for potency, sterility, toxicity, pyrogens, moisture, pH, crystallinity, heat stability, the penicillin G content and specific rotation.

(ii) Such person shall submit with his request an accurately representative sample of the batch, consisting of the following:

(*a*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 600 milligrams.

Each such package shall be taken from a different part of such batch, and each shall be packaged in accordance with the requirements of paragraph (a)(2) of this section.

(iii) In connection with contemplated requests for certification of batches of another drug in the manufacture of which it is to be used, the manufacturer of a batch which is to be so used may request the Commissioner to make check tests and assays on a sample of such batch taken as prescribed by paragraph (a)(4)(ii) of this section. From the information required by paragraph (a)(4)(i) of this section may be omitted results of tests and assays not required for the batch when used in such other drugs. The Commissioner shall report to such manufacturer results of such check tests and assays as are so requested.

(b) *Tests and methods of assay—*

(1) *Potency.* Proceed as directed in § 440.80a(b)(1), except in lieu of § 440.80a(b)(1)(iv) dissolve the sample in sufficient redistilled methanol to give a concentration of 5,000 units to 10,000 units per milliliter and then dilute with sufficient 1 percent phosphate buffer, pH 6.0, to give an appropriate stock solution.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except use medium C in lieu of medium A, and medium F in lieu of medium E.

(3) *Pyrogens.* Proceed as directed in § 440.80a(b)(3), except use physiological salt solution as the diluent and inject 2 milliliters per kilogram of a solution containing 800 units per milliliter.

(4) *Toxicity.* Proceed as directed in § 440.80a(b)(4), except use physiological salt solution as the diluent and inject, in a 10-second interval, 1.0 milliliter of a solution containing 800 units per milliliter.

(5) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i).

(6) *pH.* Proceed as directed in § 440.80a(b)(5)(ii), using a saturated aqueous solution prepared by adding 300 milligrams per milliliter.

(7) *Microscopical test for crystallinity.* Proceed as directed in § 440.80a(b)(5)(iii).

(8) *Heat stability.* Proceed as directed in § 440.80a(b)(5)(iv)(*a*), except prepare the sample as follows: Dissolve the sample in 5 milliliters of redistilled methanol. Further dilute this solution with sufficient 1-percent phosphate buffer pH 6.0 to give a concentration of 2.0 milligrams per milliliter.

(9) *Penicillin G content.* Proceed as directed in § 440.74a(b)(8) using the following formula for calculating the percent of *l*-ephenamine penicillin G:

$$\text{Percent of } l\text{-ephenamine penicillin G} = \frac{N\text{-ethyl piperidine penicillin precipitate} \times 251}{\text{Weight of sample in milligrams}}$$

(10) *Specific rotation.* Accurately weigh approximately 125 milligrams of the sample in a 25-milliliter glass-stoppered volumetric flask and dissolve in about 15 milliliters of water-acetone (1+1) at 20° C. Dilute to 25 milliliters with water-acetone (1+1) at 20° C. and mix thoroughly. Transfer the solution to a 200-millimeter tube, determine the angular rotation in a suitable polarimeter, using sodium light or a 5,893 Angstrom filter, and calculate the specific rotation. The determination must be completed within ½ hour from the time the solution is prepared.

§ 440.66 Hydrabamine penicillin G (hydrabamine penicillin G salt).

(a) *Requirements for certification—*

(1) *Standards of identity, strength, quality, and purity.* Hydrabamine penicillin G is the crystalline salt N,N'-bis-(dehydroabietyl) ethylenediamine dipenicillin G. It contains not less than 85 percent by weight of the hydrabamine sale of penicillin G. It is so purified and dried that:

(i) Its potency is not less than 845 units per milligram.

(ii) It is nontoxic.

(iii) Its moisture content is not more than 2 percent.

(iv) Its pH in a saturated aqueous solution is not less than 4.0 and not more than 7.5.

(v) Its extinction coefficient $E^{1\%}_{1cm.}$ is not less than 8 at 276 millimicrons.

(2) *Packaging.* In all cases the immediate container shall be a tight container as defined by the U.S.P. and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused that are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* Each package shall bear on its outside wrapper or container

and the immediate container, as hereinafter indicated, the following:

(i) The batch mark.

(ii) The number of units per milligram and the number of grams in the immediate container.

(iii) The statement "Expiration date ---------", the blank being filled in with the date that is 24 months after the month during which the batch was certified.

(iv) The statement "For use in the manufacture of nonparenteral drugs only".

(v) The statement "Caution: Federal law prohibits dispensing without prescription".

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in the batch, and the date on which the latest assay of the drug comprising such batch was completed. Such request shall be accompanied or followed by the results of tests and assays made by him on the batch for potency, toxicity, moisture, pH, crystallinity, the penicillin G content, and extinction coefficient.

(ii) Such person shall submit with his request an accurately representative sample of the batch, consisting of nine packages, each containing approximately 300 milligrams, plus one package containing approximately 2 grams. Each sample shall be taken from a different part of such batch and packaged in accordance with the requirement of paragraph (a)(2) of this section.

(b) *Tests and methods of assay—*

(1) *Potency.* Proceed as directed in § 440.80a(b)(1), except if the bioassay method is used prepare the sample as follows: Dissolve an accurately weighed sample in methanol and dilute with methanol to 1,000 units per milliliter (estimated). By means of a volumetric pipette, add dropwise a 1.0-milliliter aliquot to a 1,000-milliliter volumetric flask containing approximately 800 milliliters of 1-percent phosphate buffer, pH 6.0, constantly swirling the flask during the addition. Make to a volume of 1,000 milliliters with 1-percent phosphate buffer, pH 6.0. If the iodometric method is used, accurately weigh 30–50 milligrams of the sample to be tested in a 50-milliliter Erlenmeyer flask and dissolve in sufficient chloroform U.S.P. to give a concentration of 2.0 milligrams per milliliter. Pipette 2.0 milliliters of this solution into a 125-milliliter glass-stoppered Erlenmeyer flask, add 10 milliliters of 0.01*N* I_2 and titrate immediately with 0.01 *N* $Na_2S_2O_3$ for the blank determination. Toward the end of the titration add 1 drop of starch solution or about 5.0 milliliters of CCl_4. Continue the titration by the addition of 0.01–0.02-milliliter portions of 0.01*N* $Na_2S_2O_3$, shaking vigorously after each addition. The end point is reached when the blue color of the starch-iodine complex is discharged or when the CCl_4 layer becomes colorless. Prepare the solution of inactivated penicillin as follows: To 10 milliliters of the original chloroform solution add 10 milliliters of 1*N* NaOH and shake well immediately and 5 minutes later. Fifteen minutes after the initial shaking, pipette 2.0 milliliters of the upper NaOH layer into a 125-milliliter glass-stoppered Erlenmeyer flask, add 2.00 milliliters of 1.2*N* HCl and 10 milliliters of 0.01*N* I_2. Stopper the flask. After 15 minutes, titrate the excess iodine with 0.01 $Na_2S_2O_3$.

$$\text{Units of hydrabamine penicillin G per milligram} = \frac{\text{Difference in titers} \times \text{potency of F. D. A. working standard in units per milligram}}{4 \times F}$$

where F = the number of milliliters of 0.01 *N* I_2 absorbed for each 1.0 milligram of the Food and Drug Administration sodium penicillin G working standard.

(2) *Toxicity.* Accurately weigh a convenient amount of the sample (usually 1 or 2 grams) and transfer to a mortar. Add 1 drop of polysorbate 80 and, while grinding with a pestle, slowly add sterile distilled water sufficient to make a 10 percent suspension (100 milligrams per milliliter) of smooth consistency. To each of 5 mice weighing 18 grams to 25 grams each, administer orally, by means of a cannula or other suitable device, 0.5 milliliter of the 10 percent suspension. Observe the test animals for a period of 5 days for any deaths. If no animal dies within 5 days, the sample is considered nontoxic. If one or more animals die within 5 days, repeat the test, using for each test five or more previously unused mice weighing 20 grams (±0.5 gram) each; if the total deaths within 5 days is no greater than 10 percent of the total number of animals tested, including the original test, the sample is nontoxic.

(3) *Moisture.* Proceed as directed in § 440.74a(b)(5).

(4) *pH.* Proceed as directed in § 440.80a(b)(5)(ii), using a saturated aqueous solution prepared by adding approximately 60 milligrams per milliliter.

(5) *Microscopical test for crystallinity.* Proceed as directed in § 440.80a(b)(5)(iii).

(6) *Penicillin G content.* Accurately weigh a glass weighing bottle of approximately 10-milliliter capacity together with its top, a stirring rod, and a medium-porosity immersion filter stick. Transfer to the weighing bottle approximately 225 milligrams of the sample to be tested and reweigh. Add 1.5 milliliters of pyridine and stir until solution is complete. Add 0.25 milliliter of *N*-ethylpiperidine and stir until precipitation begins. When precipitation is complete, dilute with 2.5 milliliters of petroleum ether, stir well, and allow to stand for 15 minutes. Attach the filter stick to a vacuum line equipped with a suitable trap and withdraw the liquid from the precipitate. Add 2.5 milliliters of petroleum ether to the precipitate and withdraw the liquid from the precipitate. Repeat the procedure with two more 2.5-milliliter portions of petroleum ether. Leave the stirring rod and the filter stick in the bottle, dry for 30 minutes in a vacuum desiccator, stopper, and weigh.

$$\text{Percent of hydrabamine penicillin G} = \frac{\text{Milligrams of } N\text{-ethyl piperidine penicillin precipitate} \times 141.4}{\text{Weight of sample in milligrams}}$$

(7) *Absorptivity.* Accurately weigh approximately 100 milligrams of the sample, dissolve in absolute methyl alcohol, and make to 100 milliliters. Determine the absorptivity of the sample at the absorption peak at 276 mμ, using a suitable ultraviolet spectrophotometer and 1-centimeter quartz cells. Set the instrument to 100-percent transmission with absolute methyl alcohol. If a recording spectrophotometer is used, record the utraviolet absorption spectrum from 240 mμ to 300 mμ. If a nonrecording spectrophotometer is used, determine the absorbance of the solution at the 276 mμ absorption peak, using a slit width of 0.4 millimeter or less. (The exact position of the peak should be determined for the particular instrument used.) Calculate the absorptivity (1%, 1 cm.) of the sample at the absorption peak at 276 mμ.

§ 440.69 Hydrabamine phenoxymethyl penicillin.

(a) *Requirements for certification—*

(1) *Standards of identity, strength, quality, and purity.* Hydrabamine phenoxymethyl penicillin is the hydrabamine salt of phenoxymethyl penicillin. It is so purified and dried that:

(i) Its potency is not less than 825 units and not more than 965 units per milligram.

(ii) It passes the safety test.

(iii) Its moisture content is not more than 2 percent.

(iv) Its pH in a saturated aqueous solution is not less than 3.0 and not more than 6.5.

(v) Its hydrabamine absorptivity at 276 nanometers is not less than 8.

(vi) It contains not less than 48.6 percent and not more than 57 percent of phenoxymethyl penicillin.

(vii) It is crystalline.

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5 of this chapter, each package shall bear on its outside wrapper or container and the immediate container the following statement "For use in the manufacture of nonparenteral drugs only".

(3) *Requests for certification; samples.* In addition to complying with the

requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, moisture, pH, hydrabamine absorptivity, phenoxymethyl penicillin content, and crystallinity.

(ii) Samples required: 10 packages, 9 containing approximately equal portions of not less than 300 milligrams and 1 containing not less than 2 grams.

(b) *Tests and methods of assay*—(1) *Potency.* Using the phenoxymethyl penicillin working standard as the standard of comparison, assay for potency by either of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient methyl alcohol to give a solution containing 1,000 units of phenoxymethyl penicillin per milliliter (estimated). By means of a volumetric pipet, add dropwise a 1.0-milliliter aliquot of the 1,000-units-per-milliliter concentration to a 1-liter volumetric flask containing approximately 800 milliliters of 1 percent potassium phosphate buffer, pH 6.0 (solution 1), constantly swirling the flask during the addition. Dilute to volume with solution 1.

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, except in lieu of paragraph (c)(1) and (2), transfer 10 milliliters each of the sample and working standard solutions to glass-stoppered Erlenmeyer flasks, add 10 milliliters of 1*N* NaOH, and shake well immediately and 5 minutes later. Fifteen minutes after the initial shaking, pipet 2.0 milliliters of the upper NaOH layer into a 125-milliliter glass-stoppered Erlenmeyer flask and proceed as directed in paragraph (c)(3) and (4) of that section.

(2) *Safety.* Proceed as directed in § 436.33 of this chapter, except observe the mice for 5 days in lieu of 48 hours, noting mortality every 24 hours. Repeat the test as described in § 436.33(d) if one or more animals die within 5 days. Prepare the sample as follows: Accurately weigh a convenient amount of the sample (usually 1 or 2 grams) and transfer to a mortar. Add 1 drop of polysorbate 80 and, while grinding with a pestle, slowly add sufficient sterile distilled water to make a 10-percent suspension (100 milligrams per milliliter) of smooth consistency.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a saturated aqueous solution prepared by adding approximately 5 milligrams per milliliter.

(5) *Hydrabamine absorptivity.* Place an accurately weighed sample of approximately 100 milligrams into a 125-milliliter separatory funnel. Add 20 milliliters of spectrophotometric grade chloroform and 20 milliliters of 0.5*N* NaOH. Shake well for 1 minute. Allow the layers to separate, filter the lower cholorform layer through a cotton pledget, and collect the clear chloroform solution. Save the upper alkaline layer. Dilute 5 milliliters of the clear chloroform filtrate to 25 milliliters with spectrophotometric grade chloroform. Using a suitable spectrophotometer equipped with a 1-centimeter quartz cell and chloroform as the blank, determine the absorbance of each solution at 276 nanometers. (The exact position of the peak should be determined for the particular instrument used.) Calculate the hydrabamine absorptivity as follows:

$$\text{Hydrabamine absorptivity} = \frac{\text{Absorbance of sample}}{\text{Grams of sample per 100 milliliters}}$$

(6) *Phenoxymethyl penicillin content.* Dilute 5 milliliters of the alkaline layer, prepared as described in paragraph (b)(5) of this section, to 25 milliliters with distilled water. Treat a portion of the phenoxymethyl penicillin working standard in the same manner. Using a suitable spectrophotometer and 0.1*N* NaOH as the blank, determine the absorbance of each solution at 276 nanometers. Calculate the percent of phenoxymethyl penicillin as follows:

$$\text{Percent phenoxymethyl penicillin} = \frac{\text{Absorbance of sample} \times \frac{\text{Weight in milligrams of standard}}{\text{Weight in milligrams of sample}} \times \text{Percent phenoxymethyl penicillin in standard}}{\text{Absorbance of standard}}$$

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

§ 440.71 Phenoxymethyl penicillin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Phenoxymethyl penicillin is crystalline 6-(phenoxyacetamido) penicillanic acid. It contains not less than 90 percent of phenoxymethyl penicillin. It is so purified and dried that:

(i) Its potency is not less than 1,500 units per milligram.

(ii) It is nontoxic.

(iii) Its moisture content is not more than 2.0 percent.

(iv) Its pH in a saturated aqueous solution is not less than 2.5 and not more than 4.0.

(2) *Packaging.* In all cases the immediate container shall be a tight container as defined by the U.S.P. and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused that are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* Each package shall bear on its outside wrapper or container and the immediate container, as hereinafter indicated, the following:

(i) The batch mark.

(ii) The number of units per milligram and the number of grams in the immediate container

(iii) The statement "Expiration date -------", the blank being filled in with the date that is 24 months after the month in which the batch was certified, except that the blank may be filled in with the date that is 36 months or 48 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time such drug as prepared by him complies with the standards prescribed by paragraph (a)(1) of this section.

(iv) The statement "For use in the manufacture of nonparenteral drugs only".

(v) The statement "Caution: Federal law prohibits dispensing without prescription".

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in the batch, and the date on which the latest assay of the drug comprising such batch was completed. Such request shall be accompanied or followed by the results of tests and assays made by him on the batch for potency, toxicity, moisture, pH, crystallinity, and phenoxymethyl penicillin content.

(ii) Such person shall submit with his request an accurately representative sample of the batch, consisting of 10 packages, each containing approximately 300 milligrams taken from a different part of such batch, packaged in accordance with the requirements of paragraph (a)(2) of this section.

(b) *Tests and methods of assay*—(1) *Potency.* Using the phenoxymethyl penicillin working standard as the standard of comparison, assay for potency by any of the following methods; however, the results obtained from the bioassay method shall be conclusive:

(i) *Bioassay.* Proceed as directed in § 440.80a(b)(1), except prepare the sample as follows: Dissolve a weighed quantity of the sample (approximately 30 milligrams) in 2.0 milliliters of pure methanol. Further dilute this solution with sufficient 1.0 percent potassium phosphate buffer, pH 6.0, to give a concentration of 1.0 unit per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 440.80a(b)(5)(iv), except prepare the sample as follows: Dissolve a weighed quantity of the sample (approximately 30 milligrams) in 2.0 milliliters of pure methanol. Further dilute this solution with sufficient 1.0 percent potassium phosphate buffer, pH 6.0, to give a concentration of 2,000 units per milliliter (estimated).

(iii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter.

(2) *Toxicity.* Proceed as directed in § 440.80a(b)(4), except use physiological salt solution as the diluent and inject 0.5 milliliter of a solution containing 2,000 units per milliliter.

(3) *Moisture.* Proceed as directed in § 440.74a(b)(5).

(4) *pH.* Proceed as directed in § 440.80a(b)(5)(ii), using a saturated aqueous solution prepared by adding 5 milligrams per milliliter.

(5) *Microscopical test for crystallinity.* Proceed as directed in § 440.80a(b)(5)(iii).

(6) *Phenoxymethyl penicillin content.* Accurately weigh approximately 50 milligrams of the sample, dissolve in absolute methanol, and make to 100 milliliters with absolute methanol. Determine the absorbance of the sample at the absorption peak at 276 mμ, using a suitable ultraviolet spectrophotometer and quartz cells. Set the instrument to 100-percent transmission with absolute methanol. If a recording spectrophotometer is used, record the ultraviolet absorption spectrum from 240–290 mμ. If a nonrecording spectrophotometer is used, determine the absorbance (or a solution containing 20 milligrams per 100 milliliters) at the 276 mμ absorption peak, using a slit width of 0.5 millimeter or less. (The exact position of the peak should be determined for the particular instrument used.)

$$\text{Percent phenoxymethyl penicillin} = \frac{\text{Absorbance at 276 m}\mu \times 100{,}000}{\text{Milligrams of sample (per 100 milliliters)} \times a_{ss}},$$

where:

a_{ss} = the absorptivity (1%, 1 cm.) of the phenoxymethyl penicillin working standard similarly treated.

§ 440.73 Potassium phenoxymethyl penicillin (potassium phenoxymethyl penicillin salt).

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Potassium phenoxymethyl penicillin is the crystalline potassium salt of phenoxymethyl penicillin. It contains not less than 90 percent by weight of the potassium salt of phenoxymethyl penicillin. It is so purified and dried that:

(i) Its potency is not less than 1,380 units per milligram.

(ii) It is nontoxic.

(iii) Its moisture content is not more than 1.5 percent.

(iv) Its pH in a solution containing 30 milligrams per milliliter is not less than 4.0 and not more than 7.5.

(2) *Packaging; labeling; requests for certification, samples.* Proceed as directed in § 440.71(a)(2), (3) and (4).

(b) *Tests and methods of assay—*(1) *Potency.* Using the penicillin V working standard as the standard of comparison, proceed as directed in § 440.80a(b)(1).

(2) *Toxicity.* Proceed as directed in § 440.71(b)(2).

(3) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) or § 440.74a(b)(3).

(4) *pH.* Proceed as didected in § 440.80a(b)(5)(ii), using an aqueous solution prepared by adding 30 milligrams per milliliter.

(5) *Microscopical test for crystallinity.* Proceed as directed in § 440.80a(b)(5)(iii).

(6) *Phenoxymethyl pencillin content.* Accurately weigh approximately 50 milligrams of the sample, dissolve in 0.1*N* NaOH solution, and make to 100 milliliters with 0.1 *N* NaOH solution. Determine the absorbance of the sample at the absorption peak at 275 mμ using a suitable ultraviolet spectrophotometer and quartz cells. Set the instrument to 100-percent transmission with 0.1*N* NaOH solution. If a recording spectrophotometer is used, record the ultraviolet absorption spectrum from 240 mμ to 290 mμ. If a nonrecording instrument is used, determine the absorbance (of a solution containing 20 milligrams per 100 milliliters) at the 275 mμ absorption peak, using a slit width of 0.5 millimeter or less. (The exact position of the peak should be determined for the particular instrument used.)

$$\text{Percent potassium phenoxymethyl penicillin} = \frac{\text{Absorbance at 276 m}\mu \times 100{,}000 \times 100}{\text{Milligrams of sample (in 100 milliliters)} \times a_{ss} \times 90.2}$$

where:

a_{ss} = absorptivity (1% 1 cm.) of the phenoxymethyl penicillin working standard similarly treated.

90.2 = percentage of phenoxymethyl penicillin in pure potassium phenoxymethyl penicillin.

§ 440.74a Sterile procaine penicillin (penicillin procaine salt), procaine penicillin G (penicillin G procaine salt).

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Procaine penicillin is the crystalline procaine salt of a kind of penicillin, or a mixture of two or more such salts prepared from procaine hydrochloride that complies with all the standards prescribed by the U.S.P., except for the color of the crystals and penicillin, but the quantity of any salt of penicillin K therein is not more than 30 percent; procaine penicillin G is procaine penicillin which contains not less than 85 percent by weight of the procaine salt of penicillin G. Each such drug is so purified and dried that:

(i) Its potency is not less than 900 units per milligram;

(ii) It is sterile;

(iii) It is nonpyrogenic;

(iv) It is nontoxic;

(v) Its moisture content is not more than 4.2 percent; and

(vi) Its pH in saturated aqueous solution is not less than 5 and not more than 7.5.

(2) *Packaging.* In all cases the immediate containers shall be tight containers as defined by the U.S.P., shall be sterile at the time of filling and closing, shall be so sealed that the contents cannot be used without destroying the seal, and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* Each package of procaine penicillin shall bear on its outside wrapper or container and the immediate container as hereinafter indicated, the following:

(i) The batch mark;

(ii) The weight of the drug and the numbers of units in the immediate containers;

(iii) The statement "Expiration date _____", the blank being filled in with the date which is 36 months after the month during which the batch was certified, except that the blank may be filled in with the date which is 48 months or 60 months after the month during which the batch was certified, if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time such drug as prepared by him complies with the standards prescribed by paragraph (a)(1) of this section.

(iv) The statement "For manufacturing use only";

(v) The statement "Caution: Federal law prohibits dispensing without prescription".

(4) *Request for certification, check tests and assays; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch of procaine penicillin shall submi with his request a statement showing the batch mark, the number of packages of each size in the batch, the weight of the drug and the number of units in each package, and (unless it was previously submitted) the date on which the latest assay of the drug comprising the batch was completed. Such request shall be accompanied or followed by the results of tests and assays made by him on the batch for potency, sterility, toxicity, pyrogens, moisture, pH, crystallinity, penicillin K content (unless it is procaine penicillin G), and the penicillin G content if it is procaine penicillin G.

(ii) Such person shall submit with his request an accurately representative sample of the batch, consisting of the following:

(*a*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 600 milligrams.

Each such package shall be taken from a different part of such batch, and each shall be packaged in accordance with the requirements of paragraph (a)(2) of this section.

(iii) In connection with contemplated requests for certification of batches of another drug in the manufacture of which procaine penicillin is to be used, the manufacturer of a batch which is to be so used may request the Commissioner to make check tests and assays on a sample of such batch taken as prescribed by paragraph (a)(4)(ii) of this section. From the information required by paragraph (a)(4)(i) of this section may be omitted results of tests and assays not required for the batch when used in such other drug. The Commissioner shall report to each manufacturer results of such check tests and assays as are so requested.

(b) *Tests and methods of assay*—(1) *Potency.* Assay for potency by any of the following methods; however, the results obtained from the bioassay method shall be conclusive:

(i) *Bioassay.* Using the penicillin G working standard as the standard of comparison, proceed as directed in § 440.80a(b)(1).

(ii) *Iodometric assay.* Using the penicillin G working standard as the standard of comparison, proceed as directed in § 440.80a(b)(5)(iv)(*a*), except prepare the sample as follows: Dissolve a weighed sample (approximately 50 milligrams) in 2.0 milliters of pure methanol. Further dilute this solution with sufficient 1.0 percent potassium phosphate buffer, pH 6.0, to give a concentration of 2.0 milligrams per milliliter.

(iii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) or (2) of that section, except if using the method in paragraph (e)(1) of that section, add sufficient penicillinase to diluting fluid A and swirl the flask to completely solubilize the procaine penicillin before filtration. If the product contains lecithin, use diluting fluid D in lieu of A. If using the method described in paragraph (e)(2) of that section, use medium B in lieu of medium A.

(3) *Pyrogens.* Proceed as directed in § 440.80a(b)(3), except use physiological salt solution as the diluent.

(4) *Toxicity.* Proceed as directed in § 440.80a(b)(4), except use physiological salt solution as the diluent, and inject 0.5 milliliter of a solution containing 2,000 units per milliliter.

(5) *Moisture.* Proceed as directed in § 436.500(*c*), but in lieu of the directions for preparing the sample in paragraph (c)(3) thereof prepare the sample and calculate as follows: Accurately weigh about 300 milligrams of the sample into a dry titrating vessel, add an excess of the Karl Fischer reagent and back titrate with water-methanol solution until the endpoint is reached.

$$\text{Percent moisture} = \frac{(v_1 - v_2 f) \times e \times 100}{W_s}$$

where W_s = weight of sample in milligrams.

(6) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) using a saturated aqueous solution prepared by adding 300 milligrams per milliliter.

(7) *Microscopical test for crystallinity.* Proceed as directed in § 440.80a(b)(5)(iii).

(8) *Penicillin G content.* Proceed as directed in § 440.80a(b)(5)(v) using the following formula for calculating the percent of procaine penicillin G:

$$\text{Percent of procaine penicillin G} = \frac{N\text{-ethyl piperidine penicillin precipitate} \times 263.1}{\text{Weight of sample in milligrams}}$$

and in lieu of the first four sentences in § 440.80a(b)(5)(v)(b) proceed as follows:

Accurately weigh approximately 100 milligrams of the sample to be tested in a glass test tube or glass vial of approximately 10-milliliter capacity. Add 2 milliliters of water and cool to 0° C. to 5° C. Add 2 milliliters of the amyl acetate solution and 0.5 milliliter of the phosphoric acid solution, stopper and shake vigorously for approximately 15 seconds. Add a second 0.5-milliliter portion of the phosphoric acid solution and shake vigorously again. Centrifuge to obtain a clear separation of the two layers (approximately 20 seconds). If any procaine penicillin remains undissolved, add a third portion of 0.5 milliliter of the phosphoric acid solution and repeat the shaking and centrifugation.

(9) *Penicillin K content.* Weigh from 30–35 milligrams of the sample to be tested in a glass test tube or glass vial of approximately 10-milliliter capacity. Add 2.0 milliliters of chloroform U.S.P. and cool the mixture to 0° C.–5° C. in an ice bath. Add 1.0 milliliter of cold 1–4 phosphoric acid solution, stopper and shake vigorously for 15 seconds. Centrifuge to obtain a clear separation of the layers (approximately 20 seconds). After centrifuging, remove 1.0 milliliter of the chloroform layer with a pipette or syringe equipped with a suitable needle. Immediately place the 1.0 milliliter of chloroform in a 125-milliliter separatory funnel containing 29.0 milliliters of chloroform and 15.0 milliliters of 0.3 *M* phosphate (Na_2HPO_4 and KH_2PO_4) buffer pH 6.0 at room temperature and shake for 1 minute. Allow the mixture to stand with occasional swirling to settle the droplets of chloroform until the top layer is clear (usually about 10 minutes). Draw off all but about 2 milliliters of the lower chloroform layer through a small pledget of cotton into a glass-stoppered flask. Take a 4.0-milliliter aliquot of the buffer solution remaining in the separatory funnel and a 10.0-milliliter aliquot of the chloroform solution and determine the milligrams per milliliter of penicillin in each by the iodometric assay procedure described in § 440.80a(b)(5)(iv)(*a*) using 4.0 milliliters of the 1*N* NaOH and 4.0 milliliters of the 1.2*N* HCl for the two above aliquots. Make blank determinations on the same size aliquots. Calculate the percent penicillin in the buffer layer on the basis that the sum of the penicillin found in the buffer layer and in the chloroform layer is 100 percent. The percent penicillin K = (98.46—percent found in buffer) × 3.34.

§ 440.80a Sterile sodium penicillin (penicillin sodium, penicillin sodium salt), sterile calcium penicillin (penicillin calcium, penicillin calcium salt), sterile crystalline penicillin (crystalline penicillin sodium, crystalline penicillin sodium salt, crystalline penicillin potassium, crystalline penicillin potassium salt, crystalline penicillin G sodium, crystalline penicillin G sodium salt, crystalline penicillin G potassium, crystalline penicillin G potassium salt, crystalline penicillin O sodium, crystalline penicillin O sodium salt, crystalline penicillin O potassium, crystalline penicillin O potassium salt).

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium penicillin is the sodium salt of a kind of penicillin, or a mixture of two or more such salts; calcium penicillin is the calcium salt of a kind of pencillin, or a mixture of two or more such salts; crystalline penicillin is the heat-stable crystalline sodium or potassium salt of one or more kinds of penicillin, but the quantity of any salt of penicillin K therein is not more than 30 percent; crystalline penicillin G is crystalline penicillin which contains not less than 85 percent by weight of the sodium salt or potassium salt of penicillin G; crystalline penicillin O is crystalline penicillin which contains not less than 85 percent by weight of the sodium salt or potassium salt of penicillin O and it contains not more than 0.5 percent by weight of the sodium salt or potassium salt of pencillin G. Each such drug is so purified and dried that:

(i) Its potency is not less than 500 units per milligram, except that if it contains not less than 90 percent of a salt of penicillin X its potency is not less than 350 units per milligram:

(ii) It is sterile;

(iii) It is nontoxic;

(iv) It is nonpyrogenic;

(v) Its moisture content is not more than 2.5 percent unless it is crystalline

penicillin in which case its moisture content is not more than 1.5 percent;

(vi) Its pH in an aqueous solution of 30 milligrams per milliliter is not less than 5.0 and not more than 7.5.

(2) *Packaging.* In all cases the immediate containers shall be tight containers as defined by the U.S.P., shall be sterile at the time of filling and closing, shall be so sealed that the contents cannot be used without destroying the seal, and shall be of such composition as will not cause a change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded. In case it is packaged for dispensing, it shall be in immediate containers of colorless transparent glass, closed by a substance through which a hypodermic needle may be introduced and withdrawn without removing the closure or destroying its effectiveness; each such container shall contain 100,000 units, 200,000 units, 300,000 units, 500,000 units, 1,000,000 units, 2,000,000 units, 3,000,000 units, 4,000,000 units, or 5,000,000 units, except that when packaged and labeled solely for dental use each such container may contain not less than 10,000 units and except that when packaged and labeled for intravenous infusion only each such container may contain 20,000,000 units, and each may be packaged in combination with a container of the solvent, water for injection U.S.P., dextrose injection 5 percent U.S.P. (if not packaged for dental use), sodium chloride solution U.S.P. or an aqueous solution of a suitable local anesthetic.

(3) *Labeling*—(i) *It is packaged for dispensing.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its label or labeling, as hereinafter indicated, the following:

(*a*) On the outside wrapper or container and the immediate container, the statement "Expiration date _____", the blank being filled in with the date that is 18 months, or if it is crystalline penicillin, 36 months, after the month during which the batch was certified; except that, if it is crystalline penicillin, the blank may be filled in with the date that is 48 months or 60 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time such drug as prepared by him complies with the standards prescribed by paragraph (a) (1) of this section.

(*b*) On the immediate container, the statement "Sterile solution may be kept in refrigerator for __ days without significant loss of potency", the blank being filled in with the figure "3" if it is crystalline penicillin or with the figure "7" if it is not crystalline penicillin, unless such information is contained in the labeling within the package from which it is dispensed.

(*c*) On the outside wrapper or container, if it is not crystalline penicillin, the statement "Store in refrigerator not above 15° C. (59° F.)".

(ii) *It is packaged solely for manufacturing use and/or repacking.* Each package shall bear on its outside wrapper or container and the immediate container the following:

(*a*) The number of units of penicillin per milligram or per gram and the number of grams or kilograms in the immediate container.

(*b*) The statement "Caution: Federal law prohibits dispensing without prescription".

(*c*) The statement "For manufacturing use," "For repacking," or "For manufacturing use or repacking".

(*d*) The information required by paragraph (a) (3) (i) (*a*) and (*c*) of this section.

(4) *Request for certification, check tests and assays; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in the batch, the number of units in each package, and (unless it was previously submitted) the date on which the latest assay of the drug comprising the batch was completed. Such request shall be accompanied or followed by the results of tests and assays made by him on the batch for potency, sterility, toxicity, pyrogens, moisture, pH, penicillin K content (unless it is crystalline penicillin G or crystalline penicillin O), crystallinity and heat stability if it is crystalline penicillin, the penicillin G content if it is crystalline penicillin G or crystalline penicillin O, and the penicillin O content if it is crystalline penicillin O. If such batch or any part thereof is to be packaged with a solvent such request shall also be accompanied by a statement that such solvent conforms to the requirements prescribed therefor by this section.

(ii) If such batch is packaged for dispensing, such person shall submit with his request a sample consisting of the following:

(*a*) For all tests except sterility: One immediate container for each 5,000 containers in the batch, but in no case less than the following number of containers:

(*1*) If it is not crystalline penicillin, six.

(*2*) If it is crystalline penicillin, but it is not crystalline penicillin G or crystalline penicillin O, eight.

(*3*) If it is crystalline penicillin G or crystalline penicillin O, ten.

(*4*) If it is packaged in containers of less than 100,000 units each for dental use, 20, if it is not crystalline penicillin; and 40 if it is crystalline penicillin.

Such samples shall be collected by taking single immediate containers, before or after labeling, at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(*b*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation or 40 immediate containers if each contains less than 600 milligrams.

(iii) If such batch is packaged for repackaging or for use in the manufacture of another drug, such person shall submit with his request an accurately representative sample of the batch, consisting of the following:

(*a*) For all tests except sterility: Six packages; or in the case of crystalline penicillin, 10 packages, containing approximately equal portions of not less than 60 milligrams each.

(*b*) For sterility testing: 20 packages each containing approximately 600 milligrams.

Each such portion shall be taken from a different part of such batch and each shall be packaged in accordance with the requirements of paragraph (a) (2) of this section.

(iv) In connection with contemplated requests for certification of repacked batches or batches of another drug in the manufacture of which it is to be used, the manufacturer of a batch which is to be so repacked or used may request the Commissioner to make check tests and assays on a sample of such batch taken as prescribed by paragraph (a) (4) (iii) of this section. From the information required by paragraph (a) (4) (i) of this section may be omitted results of tests and assays not required for the batch when used in such other drug. The Commissioner shall report to such manufacturer results of such check tests and assays as are so requested.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Cylinders (cups).* Use stainless steel cylinders with an outside diameter of 8 mm. (±0.1 mm.), an inside diameter of 6 mm. (±0.1 mm.), and a length of 10 mm. (±0.1 mm.)

(ii) *Culture media.* Use ingredients that conform to the standards prescribed by the U. S. P. or N. F.

(*a*) Make nutrient agar for the seed layer and for carrying the test organism as follows:

Peptone	6.0 gm.
Pancreatic digest of casein	4.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Glucose	1.0 gm.
Agar	15.0 gm.
Distilled water, q. s.	1,000.0 ml.

pH 6.5 to 6.6 after sterilization.

(*b*) Make nutrient agar for the base layer as follows:

Peptone	6.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Agar	15.0 gm.
Distilled water, q. s.	1,000.0 ml.

pH 6.5 to 6.6 after sterilization.

(*c*) Make nutrient broth, for preparing an inoculum of the test organism, as follows:

Peptone	5.0 gm.
Yeast extract	1.5 gm.
Beef extract	1.5 gm.
Sodium chloride	3.5 gm.
Glucose	1.0 gm.
Dipotassium phosphate	3.68 gm.
Potassium dihydrogen phosphate	1.32 gm.
Distilled water, q.s.	1,000.0 ml

pH 7.0 after sterilization.

In lieu of preparing the media from the individual ingredients specified in paragraph (b) (1) (ii) (*a*), (*b*), and (*c*) of this section, they may be made from a dehydrated mixture which, when reconstituted with distilled water, has the same composition as such media. Minor modification of the individual ingredients specified in paragraph (b) (1) (ii) (*a*), (*b*), and (*c*) of this section are permissible if the resulting media possess growth-promoting properties at least equal to the media described.

(iii) *Working standard.* Keep the working standard (obtained from the Food and Drug Administration) at room temperature in tightly stoppered vials, which in turn are kept in larger stoppered tubes containing a suitable desiccant. Weigh out carefully in an atmosphere of 50 percent relative humidity or less between 4 and 5 milligrams of the working standard and dilute with sterile 1 percent phosphate buffer (pH 6.0) to make a stock solution of any convenient concentration. Keep this solution under refrigeration and use for 2 days only. From this stock solution make appropriate working dilutions.

(iv) *Preparation of sample.* Dissolve the sample to be tested in 1.0 percent phosphate buffer, pH 6.0, to make an appropriate stock solution.

(v) *Preparation of plates.* Add 21 ml. of agar to each Petri dish (20 x 100 millimeters). Distribute the agar evenly in the plates and allow it to harden. Use the plates the same day they are prepared. The test organism is *Staphylococcus aureus* (American Type Culture Collection 6538P).[3] Maintain the test organism on agar slants and transfer to a fresh agar slant about once a week. Prepare an inoculum for the plates by transferring the culture from the agar slant into broth and incubate at 32° C.–35° C. from 16 to 24 hours thereafter add 2.0 milliliters of this broth culture to each 100 milliliters of agar which has been melted and cooled to 48° C. Mix the culture and agar thoroughly and add 4 milliliters to each of the plates containing the 2.0 milliliters of the uninoculated agar. Tilt the plates back and forth to spread the inoculated agar evenly over the surface. Porcelain covers glazed on the outside are used. Place four cylinders on the agar surface so that they are at approximately 90° intervals on a 2.8 cm. radius. In so placing the cylinders drop them from a height of ½ inch, using a mechanical guide or device. A suspension of the test organism may be used in place of the broth culture described above in preparing the inoculum for the seeding of plates. Prepare such suspension as follows: Wash the organisms from an agar slant which has been incubated for 24 hours at 32° C.–35° C. and stored for 24 hours at room temperature with 2.0 milliliters of sterile physiological saline onto a large agar surface such as that provided by a Roux bottle containing 300 milliliters of agar. Spread the suspension of organisms over the entire agar surface with the aid of sterile glass beads. Incubate 24 hours at 32° C.–35° C. and store for 24 hours at room temperature. Wash the resulting growth from the agar surface with about 50 milliliters of sterile physiological saline. Standardize this suspension by determining the dilution which will permit 20 percent light transmission through a filter at 6,500 Angstrom units in a photoelectric colorimeter. (In the preparation of the suspensions of the test organism on agar slants and Roux bottles, the 24-hour storage periods at room temperature may be omitted if the suspensions will permit 20-percent light transmission through a filter at 6,500 Angstrom units in a photoelectric colorimeter). Determine by appropriate tests the quantity of this resulting dilution to be added to each 100 milliliters of agar, which has been melted and cooled to 48° C., for the secondary layer that will give sharp clear zones of inhibition. The suspension may be used for 1 week.

(vi) *Assay.* Use four plates for each sample. Fill one cylinder on each plate with a 1.0 unit per milliliter dilution, and one with a 0.25 unit per milliliter dilution, of the working standard. Add the estimated dilutions of 1.0 unit per milliliter and 0.25 unit per milliliter of the sample under test to the remaining two cylinders on each plate. Carefully place the plates in racks and incubate 16 to 18 hours at 32° C.–35° C. After incubation measure the diameter of each circle of inhibition to the nearest 0.5 millimeter using a colony counter with a millimeter scale etched into the supporting glass over the light source. Other measuring devices of equal accuracy may be used.

(vii) *Estimation of potency and error.* (*a*) Use the accompanying chart (Chart 1) and nomograph (Chart 2) for estimating the potency and its error. To use the chart for estimating potency two values, namely, V and W, are required. For each plate calculate two values.

$$v=(u_L+u_H)-(s_L+s_H)$$

and

$$w=(u_H+s_H)-(u_L+s_L)$$

where s_H and s_L are the diameters of the zones of inhibition in millimeters of the 1.0 unit and 0.25 unit dilutions of the standard, respectively, and u_H and u_L refer similarly to the corresponding dilutions of the sample under test. The value V is the sum of the v values for all plates and W is the sum of the w values for all plates. To estimate the potency locate the point on the chart corresponding to the values of V and W, and the potency can be read from the radial lines on the chart.

(*b*) The error of the assay is estimated by using the nomograph which requires five values, namely, the potency, V, W, Rv, and Rw. Rv (the range of the v's) is the highest value of v minus the lowest value of v obtained from the individual plates. Similarly, Rw is the difference between the highest and lowest w values. After obtaining these five values, connect with a straightedge the points corresponding to v and w on the respective scales on the right side of the nomograph. Mark with a pin or sharp-pointed pencil the intersection of the straightedge and the diagonal line of the nomograph. Move the straightedge so that it connects the value of Rw on its scale and the diagonal line at the point of the pin. The value for Q is thus determined by the scale value where the straightedge crosses the line labeled "Q". T is obtained by adding the squares of Q and Rv. On the left side of the chart connect the value of T and W with the straightedge and read the value of the ratio (error of assay-potency) where the straightedge intersects the scale of values for the ratio. This value multiplied by the potency equals the percentage error of the assay. The error of the assay calculated here estimates only how closely one assayist can check himself on any given set of dilutions of unknown and standard. It does not include any errors of weighing or errors due to variations in materials or subdivisions of a lot of penicillin.

The chart for determining potency should not be used for determinations of potency lower than 50 percent or higher than 150 percent of the standard. If the potency lies outside these limits, the assay should be repeated using a higher or lower dilution. The radial lines on the chart beyond these limits permit a rough estimation of potency from as low as 5 percent to as high as 1,000 percent when low values of W are found. If the value of V or W falls outside the limits of the chart, divide both V and W by the same proper number to bring them into the range of the chart and read the potency from the radial lines as before. If 11.4 Rw is greater than W, the slope of the assay does not differ significantly from zero and the assay is invalid. (The figure 11.4 was obtained by use of Student's "t" test for determining the significance of a slope.)

In certain laboratories it has been noted that with the 4 to 1 ratio, involving concentrations of 0.25 unit for the low dose, the zone of inhibition given by this dose may either be too small for accurate reading or have edges which are poorly defined. In order to permit the use of a higher concentration of penicillin for the low dose the third of the attached charts (Chart 3) may be used in assays in which the ratio of doses is 2 to 1, i.e., the high dose (sH) is twice the low dose (sL). As in the following chart (Chart 1), if the potency lies outside the limits of 50 percent to 150 percent the assay should be repeated, using a lower or higher dilution. The potencies beyond these limits are to be used for rough estimation purposes only. These extensions can also be used for four (or more) plate assays if both V and W are divided by the same proper number to bring them into the range of the chart. The error of the assay using the ratio of doses 2 to 1 is estimated by using the nomograph (Chart 2) in the same manner as described for the 4 to 1 ratio of doses. However, the resultant error of the assay derived in this manner must be divided by 2 to give the correct error of the assay for the 2 to 1 ratio of doses.

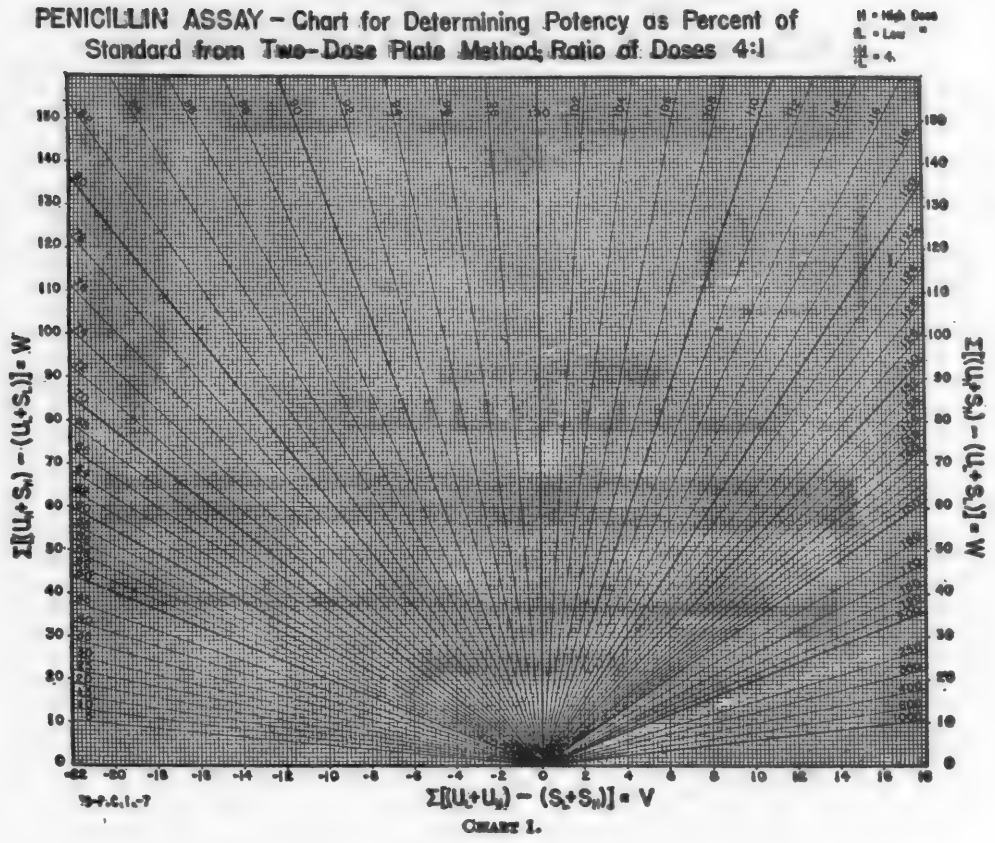
PENICILLIN ASSAY – Chart for Determining Potency as Percent of Standard from Two-Dose Plate Method; Ratio of Doses 4:1
H = High Dose
L = Low Dose
R = 4
Σ[(U1+S1) – (U2+S2)] = W
Σ[(U1+U2) – (S1+S2)] = V

CHART 1.

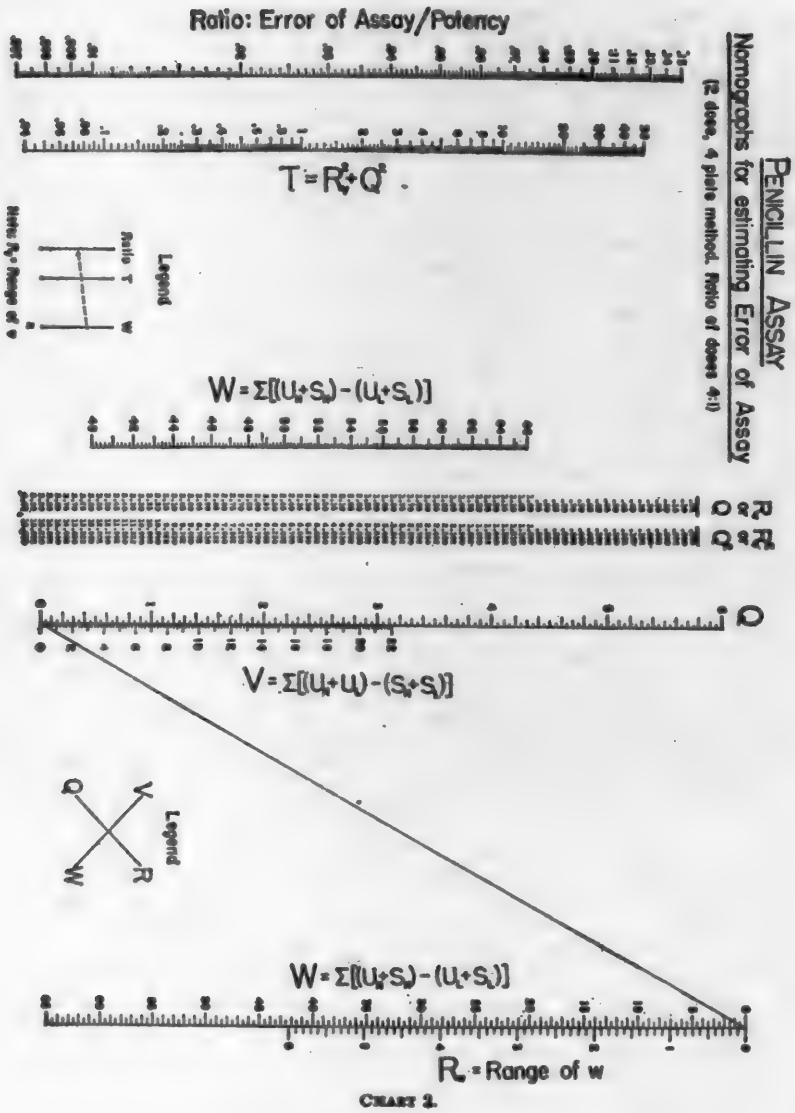
Ratio: Error of Assay/Potency
PENICILLIN ASSAY
Nomographs for estimating Error of Assay
(2 dose, 4 plate method. Ratio of doses 4:1)
T = R² + Q²
Legend
W = Σ[(U₁ + S₂) - (U₂ + S₁)]
R
Q
V = Σ[(U₁ + U₂) - (S₁ + S₂)]
W = Σ[(U₁ + S₂) - (U₂ + S₁)]
R = Range of w

CHART 2.

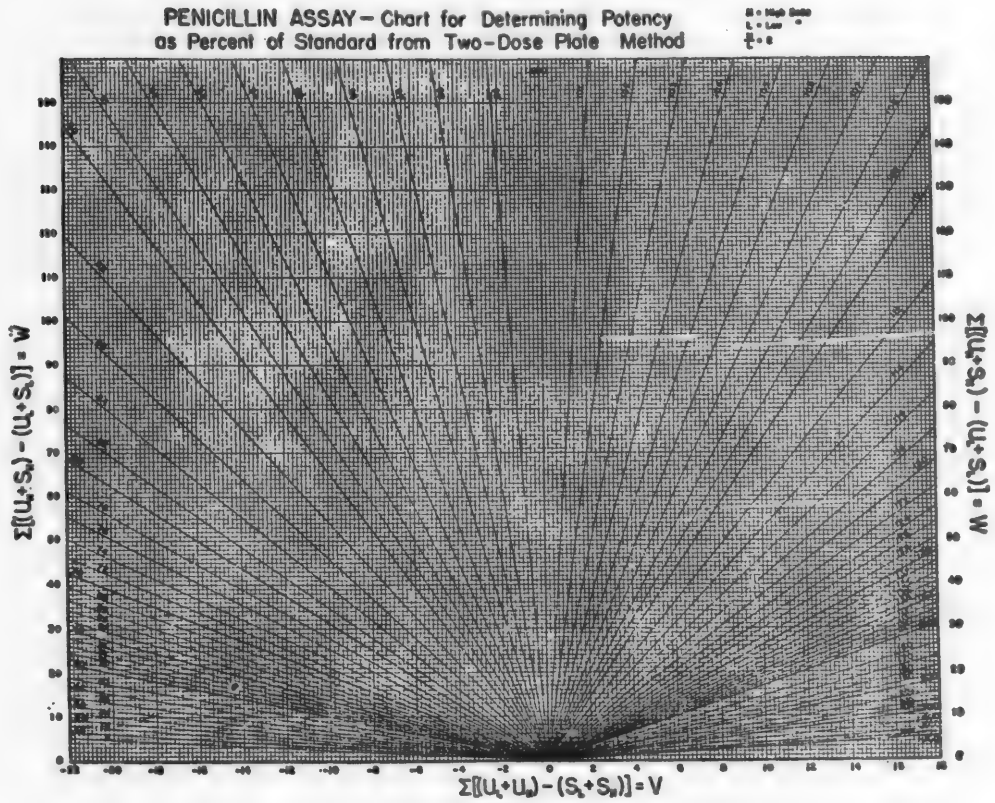
PENICILLIN ASSAY – Chart for Determining Potency as Percent of Standard from Two-Dose Plate Method
Σ[(U₁+S₂) – (U₂+S₁)] = W
Σ[(U₁+S₁) – (U₂+S₂)] = W
Σ[(U₁+U₂) – (S₁+S₂)] = V

CHART 3.

(viii) *Assay by alternative methods.* Using the penicillin G working standard as the standard of comparison, assay by any of the following methods; however, the results obtained from the bioassay method shall be conclusive. The potency of the sample may also be determined by the iodometric method as described in paragraph (b) (5) (iv) of this section or by the hydroxylamine colorimetric assay as described in § 436.205 of this chapter, or by the standard curve technique, using a single dose of standard and unknown. In the case of the standard-curve technique, dilute the sample to be tested to 1.0 unit per milliliter (estimated) in 1 percent phosphate buffer, pH 6.0. Place six cylinders on the inoculated agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius. Use three plates for each sample and fill three cylinders on each plate with the 1.0 unit per milliliter standard and three cylinders with the 1.0 unit per milliliter (estimated) sample, alternating standard and sample. Incubate the plates for 16 to 18 hours at 32° C–35° C. and measure the diameter of each circle of inhibition. Average the zone reading of the standard, and average the zone readings of the sample on the plates used. If the sample gives a larger average zone size than the average of the standard, add the difference between them to the 1.0 unit per milliliter zone size of the standard curve. If the average sample zone size is smaller than the standard value, subtract the difference between them from the 1.0 unit per milliliter zone size of the standard curve. From the curve read the concentration corresponding to these corrected values of zone sizes. Prepare concentrations for the standard curve by diluting aliquots of the standard stock solution with 1 percent phosphate buffer, pH 6.0, to give final concentrations of 0.64, 0.80, 1.0, 1.25, and 1.56 units per milliliter. Use three plates for each concentration except the 1.0 unit per milliliter concentration. Thus there will be a total of 12 plates. The 1.0 unit concentration is the reference point and is included on each plate. On each of three plates fill three cylinders with the 1.0 unit per milliliter standard and the other three cylinders with the concentration of the standard under test. Thus there will be thirty-six 1.0 unit per milliliter determinations and nine determinations for each of the other concentrations on the curve. Incubate the plates for 16 to 18 hours at 32° C.–35° C. and measure the diameter of each circle of inhibition. Average the readings of 1.0 unit per milliliter concentration and the readings of the concentration tested on each set of three plates, and average also all 36 readings of the 1.0 unit per milliliter concentration. The average of the 36 readings of the 1.0 unit per milliliter concentration is the correction point for the curve. Correct the average value obtained for each concentration to the figure it would be if the 1.0 unit per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 0.8 unit per milliliter concentration, the average of the 36 readings of the 1.0 unit per milliliter concentration is 18 millimeters and the average of the 1.0 unit per milliliter concentration on this set of three plates is 17.8 millimeters, the correction is +0.2 millimeter. If the average reading of the 0.8 unit per milliliter concentration of these same three plates is 17.0 millimeters, the corrected value is then 17.2 millimeters. Plot these corrected values including the average of the thirty-six 1.0 unit per milliliter concentrations on two-cycle semilog paper, using the concentrations in units per milliliter as the ordinate (logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5}$$

$$H=\frac{3e+2d+c-a}{5}$$

where:

L = calculated zone diameter for the lowest concentration of the standard curve,

H = calculated zone diameter for the highest concentration of the standard curve,

c = average zone diameter of 36 readings of the 1.0 unit per milliliter standard,

a, b, d, e = corrected average values for the 0.64, 0.80, 1.25, and 1.56 units per milliliter standard solutions, respectively.

Plot the values obtained for *L* and *H* and connect with a straight line.

(ix) *Potency.* The potency of sodium penicillin, calcium penicillin, and potassium penicillin is satisfactory when assayed by the methods described in this section if the immediate containers are represented to contain:

(*a*) 200,000 units or less and contain 85 percent or more of the number of units so represented;

(*b*) More than 200,000 units and contain 90 percent or more of the units so represented.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e) (1) or (2) of that section, except if using the method in paragraph (e) (2), use medium B in lieu of medium A.

(3) *Pyrogens*—(i) *Temperature recording.* Use an accurate clinical thermometer or any other temperature-recording device of equal sensitivity that has been tested to determine the time necessary to reach the maximum reading. Insert the temperature-recording device into the rectum of the test animal to a depth of not less than 7.5 centimeters and allow sufficient time to reach a maximum temperature, as previously determined, before taking the reading.

(ii) *Test animal.* Use healthy, mature rabbits, each weighing not less than 1500 grams and which have maintained their weight on an antibiotic-free diet for at least 1 week under the environmental conditions specified in this section. House the animals individually in an area of uniform temperature (±3° C. (±5° F.,) and free from disturbances likely to excite them. Do not use animals for pyrogen tests more frequently than once every 48 hours or prior to 2 weeks following their having been given a test sample that was adjudged pyrogenic. One to 3 days before using an animal that has not been used for a test during the previous 2 weeks, condition it by conducting a sham test as directed under paragraph (b) (3) (iii) of this section, omitting the injection.

(iii) *Procedure.* Perform the test in an area where the animals are housed or under similar environmental conditions. On the day of the test, withhold all food from the animals being used until after completion of the test, except that access to water may be allowed, and determine the "control temperature" of each animal. In any one test use only those animals the control temperatures of which do not deviate by more than 1° C. from each other, and do not use any animal with a temperature exceeding 39.8° C. The control temperature recorded for each rabbit constitutes the temperature from which any subsequent rise following the injection of the material is calculated. Render the syringes, needles, and glassware free from pyrogens by heating at 250° C. for not less than 30 minutes or by any other suitable method. Warm the product to be tested to approximately 37° C. Dilute the sample with sterile, pyrogen-free distilled water to a concentration of 2,000 units per milliliter. Inject 1 milliliter per kilogram into an ear vein of each of three rabbits within 30 minutes subsequent to the control temperature reading. Record the temperature at 1, 2, and 3 hours subsequent to the injection. If no rabbit shows an individual rise in temperature of 0.6° C. or more above its respective control temperature, and if the sum of the three temperature rises does not exceed 1.4° C., the sample meets the requirements for the absence of pyrogens. If one or two rabbits show a temperature rise of 0.6° C. or more, or if the sum of the temperature rises exceeds 1.4° C., repeat the test, using five other rabbits. If not more than three of the eight rabbits show individual rises in temperature of 0.6° C. or more, and if the sum of the eight temperature rises does not exceed 3.7° C., the sample meets the requirements for the absence of pyrogens.

(4) *Toxicity.* Inject intravenously each of five mice, within the weight range of 18 to 25 grams, with 0.5 milliliter of a solution of the sample prepared by diluting with sterile distilled water to approximately 4,000 units per milliliter. The injection should be made over a period of not more than 5 seconds. If no animal dies within 48 hours, the sample is nontoxic. If one or more animals die within 48 hours, repeat the test one or more times, using for each test five or more previously unused mice weighing 20 grams (±0.5 gram) each; if the total deaths within 48 hours is no greater than 10 percent of the total number of animals tested, including the original test, the sample is nontoxic.

(5) *Moisture, pH microscopical test for crystallinity*—(i) *Moisture.* In an atmosphere of about 10 percent relative humidity, transfer about 100 milligrams of the finely powdered sample to a tared weighing bottle or weighing tube equipped with a capillary-tube stopper, the capillary having an inside diameter of 0.20 millimeter–0.25 millimeter. Weigh the bottle or tube and place it in a vacuum oven without removing the stopper and dry at a temperature of 60° C. and a pressure of 5 millimeters of mercury or less for 3 hours. At the end of the drying period, fill the vacuum oven with air dried by passing it through a drying agent such as sulfuric acid or silica gel. Place weighing bottle or tube in a desiccator over a desiccating agent such as phosphorous pentoxide or silica gel, allow to cool to room temperature, and reweigh.

(ii) *pH.* Dilute the sample to be tested with carbon-dioxide-free distilled water so that the resulting solution contains 30 milligrams per milliliter. Determine the pH of this solution at 25° C. using a pH meter equipped with a glass and a calomel electrode.

(iii) *Microscopical test for crystallinity of sodium penicillin and potassium penicillin.* Mount in mineral oil and examine by means of a polarizing microscope. Crystalline penicillin shows resolvable particles which reveal the phenomena of birefringence (interference colors) and extinction positions on revolving the microscope stage. Crystalline penicillin also reveals diagnostic refractive indices when examined by the immersion method.

(iv) *Heat stability*—(*a*) *Crystalline penicillin, crystalline penicillin G.* Store a weighed sample (approximately 30 milligrams) of crystalline penicillin in an unstoppered 50-milliliter Erlenmeyer flask for 4 days in an electric oven at 100° C.±1°. At the end of this period it does not show a loss of more than 10 percent of its original potency when determined as follows: Dilute a weighed sample (approximately 30 milligrams) with a 1-percent phosphate buffer at pH 6.0 to a concentration of approximately 1.2 milligrams per milliliter (2,000 units per milliliter). Add 2.0-milliliter aliquots to each of two 125-milliliter glass-stoppered Erlenmeyer or iodine flasks. To one add 2.0 milliliters of 1*N* NaOH and allow to stand at room temperature for 15 minutes. At the end of this time add 2.0 milliliters of 1.2*N* HCl and add 10 milliliters of 0.01*N* I_2 (prepared from 0.1*N* I_2 U.S.P.). (Equal volumes of 1 *N* NaOH and 1.2 *N* HCl when mixed give pH 1.0.) After 15 minutes, titrate the excess iodine, using 0.01 *N* $Na_2S_2O_3$. Toward the end of the titration add one drop of starch solution or about 5.0 milliliters of CCl_4. Continue the titration by the addition of 0.01 to 0.02-milliliter portions of 0.01*N* $Na_2S_2O_3$, shaking vigorously after each addition. The end point is reached when the blue color of the starch-iodine complex is discharged or when the CCl_4 layer becomes colorless. To the second flask add 10 milliliters of 0.01*N* I_2 and titrate immediately with 0.01 *N* $Na_2S_2O_3$ for the blank determination. Divide the difference in titers by a factor, F, which is the number of milliliters of 0.01*N* I_2 absorbed by 1.0 milligram of sodium penicillin G working standard, to obtain the milligrams of penicillin sodium salt. Determine the factor F by actual standardization against the sodium penicillin G working standard, using the above method.

$$\text{Units of penicillin G per milligram} = \frac{\text{Difference in titers} \times \text{potency of FDA penicillin G working standard in units per milligram}}{\text{Sample weight (milligrams) in 2.0 milliliters} \times F}$$

$$\text{Percent loss of potency} = \frac{(\text{Original assay} - \text{assay after 4 days at 100° C.}) \times 100}{\text{Original assay}}$$

(*b*) *Crystalline penicillin O.* Proceed as directed in paragraph (b) (5) (iv) (*a*) of this section, except make the calculations as follows: Divide the difference in titers by a factor, *F*, which is the number of milliliters of 0.01 *N* I_2 absorbed by 1.0 milligram of the penicillin O working standard, to obtain the milligrams of potassium penicillin O. Determine the factor *F* by actual standardization against the penicillin O working standard.

$$\text{Units of penicillin O per milligram} = \frac{\text{Difference in titers} \times \text{potency of FDA penicillin O working standard in units per milligram}}{\text{Sample weight (milligrams) in 2 milliliters} \times F}$$

Penicillin O does not show a loss of more than 10 percent of its original potency.

(v) *Crystalline penicillin G*—(*a*) *Reagents.* The reagents described in paragraph (b)(5)(v)(*a*) (*1*), (2) and (3) of this section are freshly prepared every three days and are of such quality that when used in this procedure with a known penicillin G not less than 97 percent of penicillin G is recovered.

(*1*) *Amyl acetate (iso-amyl acetate) solution.* Saturate the amyl acetate (boiling range 138.5°–141.5° C.) with the *N*-ethyl piperidine salt of penicillin G by adding 2 milligrams of the salt for each 1.0 milliliter of the solvent. Cool this solution to 0°–8° C. and filter it through a sintered-glass filter immediately before use.

(2) *Acetone solution.* Saturate reagent grade acetone with the *N*-ethyl piperidine salt of penicillin G using 3 mg. of salt for each 1 milliliter of acetone. Cool this solution to 0°–8° C. and filter it through a sintered-glass filter immediately before use.

(3) *N-Ethyl piperidine solution. N*-ethyl piperidine (boiling range 129.5°–131.0° C.) should be stored in brown bottles in a refrigerator. Dilute 1.0 milliliter of this reagent with 4.0 milliliters of amyl acetate. Saturate this solution with the *N*-ethyl piperidine salt of penicillin G, using about 3 milligrams of the salt for each 1.0 milliliter of solution. Cool this solution to 0°–8° C. and filter it through a sintered-glass filter immediately before use.

(*4*) *Phosphoric acid solution.* Prepare by dissolving 1.0 milliliter of reagent grade phosphoric acid (85 percent) in 4.0 milliliters of water. Cool to 0° to 8° C. and shake before using.

(*5*) *Silica gel.* Use dry silica gel (mesh size 6–16, Tyler standard). Place about 0.5 gram of the silica gel in a micro filter funnel (approximately 10-millimeter diameter) having a fritted-glass disc of medium porosity.

(*b*) *Procedure.* Accurately weigh from 60 to 70 milligrams of the sample to be tested in a glass test tube or glass vial of approximately 10 milliliters capacity. Add 2.0 milliliters of water to dissolve the penicillin and cool the solution to 0° to 5° C. Add 2 milliliters of the amyl acetate solution and 0.5 milliliter of the phosphoric acid solution, stopper and shake vigorously for approximately 15 seconds. Centrifuge to obtain a clear separation of the two layers (approximately 20 seconds). After centrifuging, remove as much of the amyl acetate layer as possible (usually about 1.7 milliliters–1.8 milliliters) with a 2-milliliter hypodermic syringe equipped with a suitable needle, and add it to the filter funnel containing the silica gel. Allow the amyl acetate to remain in contact with the silica gel for exactly 20 seconds, then apply suction and collect the filtrate in a small test tube placed in a suction flask surrounded by cracked ice. Pipette a 1.0-milliliter aliquot of the amyl acetate filtrate into a tared flat bottom glass tube (approximately 15 x 50 millimeters) containing 1.0 milliliter of the acetone solution and 0.5 milliliter of the *N*-ethyl piperidine solution. The time elapsing between acidification and the addition of the filtrate to the above reagents should not be more than 3 minutes. Place the glass tube containing this mixture in a large weighing bottle, stopper the bottle and allow to stand for not less than 2 hours in a refrigerator at 0° to 8° C. Remove the liquid from the precipitate by means of a tared micro filter stick and wash with a total of 1 milliliter of the acetone solution adding the latter by means of a hypodermic syringe equipped with a fine needle. Place the filter stick inside the glass tube, dry under vacuum at room temperature for not less than 1 hour, and weigh. (Save all *N*-ethyl piperidine penicillin G residues for saturating reagents.)

$$\text{Percent of sodium penicillin G} = \frac{\text{mg. } N\text{-ethyl piperidine penicillin precipitate} \times 159.3}{\text{Weight of sample mg.}}$$

$$\text{Percent of potassium penicillin G} = \frac{\text{mg. } N\text{-ethyl piperidine penicillin precipitate} \times 166.5}{\text{Weight of sample mg.}}$$

(vi) *Penicillin K content.* Determine the content of penicillin K by the following method: Dilute a weighed sample or the contents of a vial with 0.3 *M* phosphate (Na_2HPO_4 and KH_2PO_4) buffer pH 6.0 to give a solution containing approximately 1,000 units/ml. In the case of calcium penicillin where a precipitate of calcium phosphate occurs, remove the precipitate by filtration and use the clear filtrate. Place a 15.0-milliliter aliquot of this solution in a 125-milliliter separatory funnel, add 30.0 milliliters of chloroform U.S.P. and shake for 1 minute. (Carry out all operations at room temperature.) Allow the mixture to stand with occasional swirling to settle the droplets of chloroform until the top layer is clear (usually about 10 minutes). Draw off all but about 12 milliliters of the lower chloroform layer through a small pledget of cotton into a glass-stoppered flask. Take a 4.0-milliliter aliquot of the original solution, a 4.0-milliliter aliquot of the buffer solution remaining in the separatory funnel and a 10.0-milliliter aliquot of the chloroform solution and determine the mg./ml. of penicillin in each by the iodometric assay procedure described in paragraph (b)(5)(iv) of this section using 4.0 milliliters of the 1 *N* NaOH and 4.0 milliliters of the 1.2 *N* HCl for each of the above aliquots. Make blank determinations on the same size aliquots. Calculate the percent penicillin in the buffer layer and in the chloroform layer as compared to the original solution. The sum of these percentages should be 100%±2%. The percent penicillin K=(96.92+% in chloroform—% in buffer)×1.67. (The factors in the above formula are based on distribution coefficients of penicillin K and G between chloroform and aqueous phosphate buffer at pH 6.0.)

(vii) *Penicillin G content of crystalline penicillin O.* Accurately weigh approximately 300 milligrams of the sample to be tested in a 250-milliliter Erlenmeyer flask, dissolve in 1.0 milliliter of 2.5 percent KOH solution, add with swirling 50.0 milliliters of 4.5 percent $KMnO_4$ solution and heat on the steam bath for 2 hours, covering the flask with a watch glass. Cool to room temperature and add 25.0 milliliters of 10 percent oxalic acid solution. When the reaction has ceased, add 15.0 milliliters of 18 *N* H_2SO_4 stepwise with agitation and cooling. Add 30.0 grams of NaCl and agitate until a clear, colorless solution is obtained. Transfer to a separatory funnel and extract with three portions of chloroform, 30 milliliters, 20 milliliters, and 10 milliliters in that order. Filter the extracts through a pledget of cotton previously moistened with chloroform and collect in a second separatory funnel. Add 50.0 milliliters of 0.1*N* NH_4OH to the combined extracts, shake 5 minutes and allow the phases to separate for 10 minutes. Discard all but a few milliliters of the chloroform layer. Draw off the last few milliliters of chloroform and about 15.0 milliliters of the NH_4OH layer into a glass-stoppered test tube and centrifuge 2 minutes to obtain a clear aqueous layer. Prepare a blank by shaking 50.0 milliliters of 0.1*N* NH_4OH with 60.0 milliliters of chloroform as described above. Determine the absorbance of the blank compared with 0.1*N* NH_4OH at 220 mμ and 224 mμ. Determine the absorbance of the sample at 220 mμ and 224 mμ compared with the blank. Calculate the quantity of benzoic acid in the solution from the equation:

$$x = \frac{R(A224) - (A220)}{RB - A}$$

where:

x = concentration of milligrams per milliliter of benzoic acid in sample.
A = absorbance per milligram of benzoic acid U.S.P. per milliliter of 0.1 *N* NH_4OH at 220 mμ.
B = absorbance per milligram of benzoic acid U.S.P. per milliliter of 0.1 *N* NH_4OH at 224 mμ.
R = absorbance of blank at 220 mμ / absorbance of blank at 224 mμ.
$A220$ = absorbance of sample at 220 mμ.
$A224$ = absorbance of sample at 224 mμ.

Calculate the quantity of penicillin G in the sample from the equation:

$$\frac{3.05 \times (50)\,(100)}{\text{Weight of sample in milligrams}} = \text{percent penicillin G.}$$

(viii) *Penicillin O content.* (a) Accurately weigh approximately 100 milligrams of the sample in a 25-milliliter glass-stoppered test tube and add 5.0 milliliters of chloroform (previously washed with water) and 15 milliliters of distilled water. Place the tube in an ice bath for 5 minutes and then add 1.5 milliliters of 1 to 4 H_2PO_4. Shake the tube vigorously for 2 minutes, centrifuge for 1 minute to separate the layers, and withdraw the lower chloroform layer

with the aid of a 10-milliliter hypodermic syringe equipped with a 3-inch needle. Superficially dry the chloroform by filtering through a pledget of cotton, using a U-shaped funnel to reduce evaporation during filtration. Collect the filtrate in a 5-milliliter glass-stoppered bottle and use within an hour. Place this chloroform solution in the absorption cell, which consists of two rock-salt plates with a 1.0-millimeter polyethylene spacer between them, clamped firmly in the cell holder. Adjust the amplification of a suitable infrared spectrometer to full-scale deflection for 1 μv., set the slit opening at 0.300 millimeter, and record the spectrum from 10.7 μ to 9.4 μ, taking a zero reading (shutter closed) at the beginning and at the end of the run. Calculate the baseline absorbance from the following equation:

$$A_b = \text{Log}_{10}\frac{I_b}{I_p}$$

where:

baseline absorbance.

A_b = distance from the zero line to the transmission peak at 10.3 μ.

I_p = distance from the zero line to the maximum absorption of the band at 10.1 μ.

The absorptivity, a, of the sample in the particular cell being used is calculated as follows:

$$a = \frac{A_b}{\text{Weight of sample}}$$

Using an accurately weighed sample of about 100 milligrams of the potassium penicillin O working standard in the above procedure, determine its absorptivity in the same cell. Obtain the percent penicillin O in the sample under test by the following calculation:

$$\frac{a\ (\text{sample})}{a\ (\text{standard})} \times 100 = \text{percent potassium penicillin O.}$$

$$\frac{a\ (\text{sample})}{a\ (\text{standard})} \times 95.6 = \text{percent sodium penicillin O.}$$

(*b*) If the sample is potassium penicillin O, the following method may be used: Grind the sample to a uniform powder, using a mortar and pestle. Weigh, by difference, 100 milligrams to 150 milligrams of liquid petrolatum into an agate mortar. Divide the actual weight of the liquid petrolatum by three, and add exactly this amount of the powdered penicillin O to the liquid petrolatum in the mortar. Mix with a small spatula and then mull thoroughly with the pestle until a uniform consistency is obtained. Use two circular rock-salt plates, each 2 inches in diameter, as the absorption cell. Place a small drop of the mull in the center of one of the rock-salt plates. Place a brass spacer, 0.0036-thick, on the plate. (This spacer is cut in the shape of a circular gasket with a 1-inch center hole and a slit to permit the escape of air when the two plates are pressed together.) Put on the top salt plate gently and slowly squeeze together to spread the mull uniformly. Clamp the two plates firmly together in a metal cell holder. (The cell holder consists of two metal plates, one containing a rectangular center slit ¼-inch wide x ⅝-inch long, the other with a center hole 1 inch in diameter. The two plates are clamped together by means of threaded studs and nuts.) Examine the assembled cell by holding it up to the light. It should appear smooth, free of any air bubbles, and not in contact with the spacer. Place the cell in a suitable infrared spectrophotometer. Adjust the amplification of the spectrometer to full-scale deflection for 1μ v., set the slit opening to about 0.300, and run the spectrum from 9.4μ to 10.7μ, using an automatic slit-control mechanism and taking a zero reading (shutter closed) at the beginning and at the end of the run. Draw a baseline between two points, one on each side of the analytical band (10.1μ), and calculate the baseline absorbance, using the following equation:

$$A_b = \log_{10}\frac{I_b}{I_p}$$

where:

I_p = baseline absorbance.

A_b = distance from the zero line to the maximum absorption of the band.

I_b = distance from the zero line to the baseline, measured at the same wavelength as I_p.

Using known mixtures of penicillin G working standard and penicillin O working standard, prepare a standard curve by plotting the baseline absorbance values obtained against the percent penicillin O. Obtain the percent penicillin O in the sample under test from this standard curve.

(6) *Penicillin X.* Dissolve the contents of a 100,000 unit ampul in about 20 milliliters of ice cold distilled water. Transfer quantitatively to a 100-milliliter volumetric flask, rinsing the ampul with small portions of ice cold water and make to 100 milliliters. Pipette a 50.0-milliliter aliquot into a 125-milliliter separatory funnel, then add 50.0 milliliters of cold chloroform and shake the mixture. Add an amount of approximately 1*N* H_2SO_4 to bring the pH of the aqueous layer to 2.0. (The amount of 1*N* H_2SO_4 to be added is calculated by titrating a separate 5.0-milliliter aliquot of the 100-milliliter dilution to pH 2.0 using a suitable pH meter.) Shake the mixture vigorously for one minute. Allow the layers to separate and filter the chloroform through a small pledget of cotton, moistened with chloroform, into a second 125-milliliter separatory funnel. Shake the acid aqueous solution with a second 50.0 milliliters of cold chloroform and, when the layers have separated, withdraw the chloroform through the same filter into the second separatory funnel. Immediately neutralize the acid aqueous solution, containing the penicillin X, with 0.1 *N* NaOH to pH 6.5 to 7.0 using the pH meter and make to 100 milliliters with water. Make appropriate dilutions in 1 percent phophate buffer at pH 6.0 and assay as directed in paragraph (b)(1)(vi) or (viii) of this section. Shake the combined chloroform extracts, containing any penicillin G, etc., with small successive portions of cold $NaHCO_3$ solution (0.1 percent), until the combined $NaHCO_3$ extracts give a pH of 7.0, and make to 100 milliliters with water. Make the proper estimated dilutions in 1 percent phosphate buffer at pH 6.0. Assay these last dilutions as directed in paragraph (b)(1)(vi) or (viii) of this section. The potency of the penicillin X fraction plus potency of the penicillin G, etc., fraction should approximate that of the potency of the original solution. All of the above extractions should be carried out in a cold room.

§ 440.81 Buffered crystalline penicillin.

(a) *Requirements for certification.* Buffered crystalline penicillin conforms to all requirements prescribed by § 440.80a(a) for crystalline penicillin, and is subject to all procedures prescribed by § 440.80a(a) for crystalline penicillin, except that:

(1) It contains the buffer sodium citrate in a quantity not less than 4.0 percent and not more than 5.0 percent by weight of its total solids. It may contain citric acid in a quantity not more than 0.15 percent of its total solids in place of a corresponding amount of sodium citrate. Such sodium citrate and citric acid conform to the standards prescribed therefor by the U.S.P.;

(2) If it is crystalline penicillin G, the penicillin G content is corrected for the sodium citrate content;

(3) The immediate container, if it is packaged for dispensing, shall bear, in lieu of the statement prescribed for crystalline penicillin by § 440.80a(a)(3)(i)(b), the statement "Sterile solution may be kept in refrigerator for 1 week without significant loss of potency", unless such information is contained in the circular or other labeling within or attached to the package from which it is dispensed;

(4) A person who requests certification of a batch shall submit with his request a statement showing the quantity of sodium citrate used in making the batch, that such sodium citrate conforms to the requirements prescribed therefor by this section, and in case of an initial request for certification he shall submit an accurately representative sample of such sodium citrate consisting of approximately 5 grams.

(b) *Tests and methods of assay.* Proceed as directed in § 440.80a(b)(1), (2), (3), (4), (5) and (6).

§ 440.82 Potassium phenethicillin.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Potassium phenethicillin is the DL-α-phenoxyethyl penicillin potassium salt. It is so purified and dried that:

(i) Its potency is not less than 1,328 units per milligram.

(ii) It passes the safety test.

(iii) Its loss on drying is not more than 1.5 percent.

(iv) Its pH in an aqueous solution of 5,000 units to 10,000 units per milliliter is not less than 4.0 and not more than 7.5.

(v) Its phenethicillin content is not less than 81.5 percent.

(vi) It contains not less than 55 percent and not more than 75 percent of L-phenethicillin potassium.

(vii) It is crystalline.

(viii) It passes the identity test.

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5(b) of this chapter, each package shall bear on its outside wrapper or container and the immediate container the following statement "For use in the manufacture of nonparenteral drugs only".

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, loss on drying, pH, phenethicillin content, L-phenethicillin potassium content, crystallinity, and identity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Total potency.* Assay for total potency by either of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(i) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter.

(ii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter.

(2) *Safety.* Proceed as directed in § 436.33 of this chapter.

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 5,000 units to 10,000 units per milliliter.

(5) *Phenethicillin content.* Accurately weigh approximately 50 milligrams each of the sample and L-phenethicillin working standard into separate 100-milliliter volumetric flasks. Dissolve and dilute to volume with distilled water. Using a suitable spectrophotometer equipped with 1-centimeter quartz cells and distilled water as the blank, set the instrument to 100 percent transmission and then determine the absorbance of each solution at the absorbance peak at 268 nanometers. Calculate the percent phenethicillin as follows:

$$\text{Percent phenethicillin} = \frac{\text{Absorbance of sample} \times \text{weight of standard in milligrams} \times \text{percent L-phenethicillin content of standard}}{\text{Absorbance of standard} \times \text{weight of sample in milligrams}}$$

(6) *L-phenethicillin potassium content*—(i) *Microbial assay of L-phenethicillin potassium equivalent (microbiological agar diffusion assay).* Using the L-phenethicillin working standard, proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to obtain an activity estimated to be equivalent to 0.1 unit of L-phenethicillin per milliliter.

(ii) *L-phenethicillin equivalent of the D-phenethicillin working standard.* Using the L-phenethicillin working standard as the standard of comparison, proceed as directed in § 436.105 of this chapter, preparing the sample (D-phenethicillin working standard) for assay as follows: Dissolve an accurately weighed portion of D-phenethicillin working standard in sufficient sterile distilled water to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to obtain an activity estimated to be equivalent to 0.1 unit of L-phenethicillin per milliliter.

(iii) *Calculation.* Calculate the L-phenethicillin potassium content of the sample as follows:

$$\text{Percent L-phenethicillin potassium content} = \frac{(R-r)}{(1-r)} \times 100$$

where:

$$R = \frac{\text{Units of L-phenethicillin potassium equivalent (found per milligram of sample in microbial assay)}}{\text{Units per milligram found in chemical assay of sample (total potency)}};$$

$$r = \frac{\text{L-phenethicillin potassium equivalent in units per milligram (of D-phenethicillin potassium standard)}}{\text{Potency in units per milligram of L-phenethicillin standard}}$$

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(8) *Identity.* Add 0.5 ml of a methyl alcohol solution containing 8 mg per milliliter of L-phenethicillin working standard to a test tube. Add 0.5 ml of the sample solution, similarly prepared, to a second test tube. Evaporate the methyl alcohol in each test tube under a current of air. Add 0.1 ml of an aqueous chromotropic acid solution containing 10 mg per milliliter to each tube. Add 2 ml of concentrated sulfuric acid to each tube and heat in a glycerol bath at 150° C. for 3 to 4 minutes. The same green color is produced by both sample and working standard. (Phenoxymethyl penicillin or its salts give a blue or purple color.)

Subpart B—Oral Dosage Forms

§ 440.105 Ampicillin oral dosage forms.

§ 440.105a Ampicillin tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Ampicillin tablets are composed of ampicillin with one or more suitable and harmless diluents and lubricants. Each tablet contains 250 or 500 milligrams of ampicillin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of ampicillin that it is represented to contain. Its loss on drying is not more than 4 percent. The tablets disintegrate within 15 minutes. The ampicillin used conforms to the standards prescribed by § 440.5(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The ampicillin used in making the batch for potency, safety, loss on drying, pH, ampicillin content, concordance, crystallinity, and identity.

(*b*) The batch for potency, loss on drying, and disintegration time.

(ii) Samples required:

(*a*) The ampicillin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of tablets into a high-speed glass blender jar with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, preparing the sample solution as follows: Place a representative number of tablets in a high-speed glass blender jar and add sufficient distilled water to give a convenient concentration. Blend for 3 to 5 minutes. Further dilute an aliquot with distilled water to the prescribed concentration.

(2) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter, using the procedure described in paragraph (e)(1) of that section.

§ 440.105b Ampicillin chewable tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Each ampicillin chewable tablet contains 125 milligrams or 250 milligrams of ampicillin with suitable binders, lubricants, flavorings, and colorings. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of ampicillin that it is represented to contain. Its loss on drying is not more than 3 percent. The ampicillin used conforms to the standards prescribed by § 440.5(a)(1).

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5 of

this chapter, this drug shall be labeled "ampicillin tablets".

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) The results of tests and assays on:

(*a*) The ampicillin used in making the batch for potency, safety, loss on drying, pH, ampicillin content, concordance, crystallinity, and identity.

(*b*) The batch for potency and loss on drying.

(ii) Samples required:

(*a*) The ampicillin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of tablets into a high-speed glass blender jar with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, preparing the sample as follows: Blend a representative number of tablets in a high-speed blender with sufficient distilled water to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with distilled water to the prescribed concentration.

(2) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter.

Effective date: § 440.105b became effective on April 15, 1974, except that the nonproprietary name designated in paragraph (a)(2) shall not be effective until new labels are printed in the normal course of business or until September 15, 1974, whichever comes first. See 39 FR 13877, April 18, 1974.

§ 440.105c Ampicillin capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Ampicillin capsules are composed of ampicillin with or without one or more buffer substances, diluents, binders, lubricants, vegetable oils, colorings, and flavorings, enclosed in a gelatin capsule. Each capsule contains 125 milligrams, 250 milligrams, or 500 milligrams of ampicillin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of ampicillin that it is represented to contain. The loss on drying is not more than 4.0 percent. The ampicillin used conforms to the standards prescribed by § 440.5(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The ampicillin used in making the batch for potency, safety, loss on drying, pH, ampicillin content, concordance, crystallinity, and identity.

(*b*) The batch for potency and loss on drying.

(ii) Samples required:

(*a*) The ampicillin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Assay for potency by either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, preparing the sample as follows: Place the contents of a representative number of capsules into a high-speed glass blender jar, and add sufficient distilled water to give a convenient concentration. Blend for 3 to 5 minutes. Filter through Whatman No. 2 filter paper. Further dilute an aliquot of the filtrate with distilled water to the prescribed concentration.

(2) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter.

§ 440.105d Ampicillin for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Ampicillin for oral suspension is a mixture of ampicillin with one or more suitable and harmless colorings, flavorings, buffer substances, sweetening ingredients, and preservatives. When reconstituted as directed in the labeling, it contains either 25 milligrams, 50 milligrams, or 100 milligrams of ampicillin per milliliter. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of ampicillin that it is represented to contain. Its moisture content is not more than 2.5 percent. When reconstituted as directed in the labeling, its pH is not less than 5.0 and not more than 7.5. The ampicillin used conforms to the standards prescribed by § 440.5(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The ampicillin used in making the batch for potency, safety, loss on drying, pH, ampicillin content, concordance, crystallinity, and identity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The ampicillin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 6 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Assay for potency by either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute the drug as directed in the labeling. Place an accurately measured representative portion of the sample into a suitable volumetric flask and dilute to volume with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a convenient concentration. Mix well. Further dilute an aliquot with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, preparing the sample as follows: Reconstitute the drug as directed in the labeling. Place an accurately measured aliquot (usually a single dose) into an appropriate-sized volumetric flask and dilute to volume with 1 percent potassium phosphate buffer, pH 6.0 (solution 1). Mix well. Further dilute with solution 1 to the prescribed concentration.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

§ 440.107 Ampicillin trihydrate oral dosage forms.

§ 440.107a Ampicillin trihydrate chewable tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Ampicillin trihydrate chewable tablets are composed of ampicillin trihydrate with or without one or more suitable diluents, lubricants, preservatives, and flavorings. Each tablet contains ampicillin trihydrate equivalent to 125 or 250 milligrams of ampicillin. Its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of ampicillin that it is represented to contain. Its moisture content is not more than 5.0 percent. The ampicillin trihydrate used conforms to the standards prescribed by § 440.7(a)(1).

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5 of this chapter, this drug shall be labeled "ampicillin tablets."

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The ampicillin trihydrate used in making the batch for potency, safety, loss on drying, pH, ampicillin content, concordance, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The ampicillin trihydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of tablets into a high-speed glass blender jar with sufficient 0.1*M* potassium phosphate buffer, pH 6.0 (solution 3), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, preparing the sample as follows: Place a representative number of tablets into a high-speed glass blender jar containing sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a convenient concentration. Blend for 5 minutes. Further dilute with solution 1 to the prescribed concentration.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

Effective date: § 440.107a became effective on April 15, 1974, except that the nonproprietary name designated in paragraph (a)(2) shall not be effective until new labels are printed in the normal course of business or until September 15, 1974, whichever comes first. See 39 FR 13877, April 18, 1974.

§ 440.107b Ampicillin trihydrate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Ampicillin trihydrate capsules are composed of ampicillin trihydrate with or without one or more buffer substances, diluents, binders, lubricants, vegetable oils, colorings, and flavorings, enclosed in a gelatin capsule. Each capsule contains ampicillin trihydrate equivalent to 250 milligrams or 500 milligrams of ampicillin. Its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of ampicillin that it is represented to contain. Its loss on drying is not less than 10 percent and not more than 15 percent. The ampicillin trihydrate used conforms to the standards prescribed by § 440.7(a)(1).

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5 of this chapter, this drug shall be labeled "ampicillin capsules."

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The ampicillin trihydrate used in making the batch for potency, safety, loss on drying, pH, ampicillin content, concordance, crystallinity, and identity.

(*b*) The batch for potency and loss on drying.

(ii) Samples required:

(*a*) The ampicillin trihydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Assay for potency by either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, preparing the sample as follows: Place the contents of a representative number of capsules into a high-speed glass blender jar, and add sufficient distilled water to give a convenient concentration. Blend for 3 to 5 minutes. Filter through Whatman No. 2 filter paper. Further dilute an aliquot of the filtrate with distilled water to the prescribed concentration.

(2) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter.

Effective date: § 440.107b became effective on April 15, 1974, except that the nonproprietary name designated in paragraph (a)(2) shall not be effective until new labels are printed in the normal course of business or until September 15, 1974, whichever comes first. See 39 FR 13877, April 18, 1974.

§ 440.107c Ampicillin trihydrate for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Ampicillin trihydrate for oral suspension is a mixture of ampicillin trihydrate with one or more suitable and harmless colorings, flavorings, buffers, sweetening ingredients, and preservatives. When reconstituted as directed in the labeling, it contains ampicillin trihydrate equivalent to either 25, 50, or 100 milligrams of ampicillin per milliliter. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of ampicillin that it is represented to contain: Its moisture content is:

(i) Not more than 2.5 percent if it contains sugar and is intended to contain the equivalent of 25 or 50 milligrams of ampicillin per milliliter when reconstituted as directed in the labeling; or

(ii) Not more than 5 percent if it contains sugar and is intended to contain the equivalent of 100 milligrams of ampicillin per milliliter when reconstituted as directed in the labeling; or

(iii) Not more than 8 percent if it is sugarless and is intended to contain the equivalent of 25 milligrams of ampicillin per milliliter when reconstituted as directed in the labeling; or

(iv) Not more than 12 percent if it is sugarless and is intended to contain the equivalent of 100 milligrams of ampicillin per milliliter when reconstituted as directed in the labeling. Its pH, when reconstituted as directed in the labeling, is not less than 5.0 and is not more than 7.5. The ampicillin trihydrate used conforms to the standards prescribed by § 440.7(a)(1).

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5 of this chapter, this drug shall be labeled "ampicillin for oral suspension."

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The ampicillin trihydrate used in making the batch for potency, safety, loss on drying, pH, ampicillin content, concordance, crystallinity, and identity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The ampicillin trihydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Assay for potency by either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute the drug as directed in the labeling. Place an accurately measured representative portion of the sample into a suitable volumetric flask and dilute to volume with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a convenient concentration. Mix well. Further dilute an aliquot with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, preparing the sample as follows: Reconstitute the drug as directed in the labeling. Place an accurately measured aliquot (usually a single dose) into an appropriate-sized volumetric flask and dilute to volume with 1 percent potassium phosphate buffer, pH 6.0 (solution 1). Mix well. Further dilute with solution 1 to the prescribed concentration.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

Effective date: § 440.107c became effective on April 15, 1974, except that the nonproprietary name designated in paragraph (a)(2) shall not be effective until new labels are printed in the normal course of business or until September 15, 1974, whichever comes first (39 FR 13877, April 18, 1974).

§ 440.107d Ampicillin trihydrate-probenecid for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Ampicillin trihydrate and probenecid for oral suspension is a dry mixture of ampicillin trihydrate and probenecid with suitable flavorings, lubricants, colorings, and suspending agents packaged in a single dose container. When reconstituted as directed in the labeling, each single dose will contain ampicillin trihydrate equivalent to 3.5 grams of ampicillin and 1.0 gram of probenecid. Its ampicillin content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of grams of ampicillin that it is represented to contain. Its probenecid content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of grams of probenecid that it is represented to contain. Its moisture content is not more than 5.0 percent. When reconstituted as directed in the labeling, its pH is not less than 5.0 and not more than 7.5. The ampicillin trihydrate used conforms to the standards prescribed by § 440.7(a)(1). The probenecid used conforms to the standards prescribed by the U.S.P. XVIII.

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5 of this chapter, this drug shall be labeled: "ampicillin-probenecid for oral suspension".

(3) *Requests for certification, samples.* In addition to complying with the requirements for § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The ampicillin trihydrate used in making the batch for potency, safety, loss on drying, pH, ampicillin content, concordance, and identity.

(*b*) The probenecid used in making the batch for all U.S.P. XVIII specifications.

(*c*) The batch for ampicillin content, probenecid content, moisture, and pH.

(ii) Samples required:

(*a*) The ampicillin trihydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 10 immediate containers.

(b) *Tests and methods of assay*—(1) *Ampicillin content*—(i) *Sample preparation.* Reconstitute as directed in the labeling and mix well. Drain the suspension from the bottle for 30 seconds into a high-speed glass blender jar containing sufficient sterile distilled water to obtain a total volume of 500 milliliters. Blend for 10 minutes.

(ii) *Assay procedures.* Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the aqueous solution with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, diluting an aliquot of the aqueous solution to the prescribed concentration.

(2) *Probenecid content*—(i) *Preparation of standard solution.* Transfer approximately 25 milligrams of U.S.P. probenecid reference standard, accurately weighed, to a 25-milliliter volumetric flask. Dissolve and dilute to volume with 1 percent aqueous sodium carbonate solution.

(ii) *Preparation of sample solution.* Reconstitute the sample as directed in the labeling and mix well. Drain the suspension from the bottle for 30 seconds into a 1,000-milliliter volumetric flask. Dilute to volume with 1 percent aqueous sodium carbonate solution, shake well, and filter through Whatman No. 6 filter paper. Discard the first 10-milliliter portion.

(iii) *Procedure.* Transfer 2.0 milliliters of the clear filtrate to a 125-milliliter separatory funnel and add 8.0 milliliters of 1.0*N* hydrochloric acid. Extract the solution with four 20-milliliter portions of chloroform, filtering each extract through a glass wool pledget into a 100-milliliter volumetric flask. Wash the pledget with chloroform, dilute to volume with chloroform and mix. Treat 2.0 milliliters of the standard solution in the same manner. Using a suitable spectrophotometer equipped with a 1-centimeter cell and chloroform washed with 1 percent aqueous sodium carbonate solution as a blank, determine the absorbance of the sample and standard solutions at the peak near 257 nanometers.

(iv) *Calculations.* Calculate the probenecid content as follows:

$$\text{Grams probenecid per container} = \frac{\text{Absorbance of sample} \times \text{weight of standard in milligrams} \times \text{percent purity of standard}}{\text{Absorbance of standard} \times 25 \times 100}$$

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

Effective date: § 440.107d became effective on April 15, 1974, except that the nonproprietary name designated in paragraph (a)(2) shall not be effective until new labels are printed in the normal course of business or until September 15, 1974, whichever comes first. See 39 FR 13877, April 18, 1974.

§ 440.111 Carbenicillin indanyl sodium tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Carbenicillin indanyl sodium tablets are composed of carbenicillin indanyl soduim and one or more suitable and harmless diluents, binders, lubricants, colorings, and coating substances. Each tablet contains carbenicillin indanyl sodium equivalent to 382 milligrams of carbenicillin. Its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of carbenicillin that it is represented to contain. Its moisture content is not more than 2.0 percent. It gives a positive identity test for carbenicillin indanyl sodium. The tablets shall disintegrate within 1 hour. The carbenicillin indanyl sodium used conforms to the standards prescribed by § 440.11(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The carbenicillin indanyl sodium used in making the batch for potency, safety, moisture, pH, and identity.

(*b*) The batch for potency, moisture, identity, and disintegration time.

(ii) Samples required:

(*a*) The carbenicillin indanyl sodium used in making the batch: Five packages, each containing approximately 1 gram and one package containing approximately 2.5 grams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.300 of this chapter, except:

(i) *Preparation of the sample.* Accurately weigh 20 tablets and determine the average tablet weight. Using a mortar and pestle, grind the tablets to a fine powder. Accurately weigh a portion of the powder approximately equivalent to the weight of one tablet and transfer it into a 100-milliliter volumetric flask. Add approximately 70 milliliters of distilled water and shake the flask for 5 minutes. Dilute to volume and mix well. Transfer a 5-milliliter aliquot of the stock solution to a 50-milliliter glass-stoppered centrifuge tube. (The solution will be slightly turbid.) Add 15 milliliters of phosphate-citrate buffer and 20 milliliters of 4-methyl-2-pentanone to the tube. Stopper the tube and shake it for 10 seconds. Centrifuge at 2,000 revolutions per minute to separate the phases. Remove about 15 milliliters of the upper phase and proceed as directed in § 436.300(e) of this chapter.

(ii) *Calculations.* Calculate the carbenicillin content (potency) of the tablets as follows:

$$\text{Milligrams of carbenicillin per tablet} = \frac{\text{Degrees of rotation of sample solution} \times \text{weight of working standard} \times \text{average tablet weight} \times 100 \times \text{micrograms of carbenicillin in each milligram of the working standard}}{\text{Degrees of rotation of working standard} \times \text{weight of sample} \times 25 \times 1{,}000};$$

where:
100 and 25=the volume of the sample and working standard solutions, respectively;
1,000=factor to correct micrograms to milligrams.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Identity.* Proceed as directed in § 436.301 of this chapter, preparing the sample as follows: Using a mortar and pestle, grind a representative number of tablets into a fine powder. Dissolve a weighed amount of this powder in sufficient extraction solvent (described in § 436.301(b)(1) of this chapter) to give 10 milligrams of carbenicillin per milliliter. Shake the mixture for 5 minutes and promptly dilute an aliquot in extraction solvent to obtain a final concentration of 1 milligram carbenicillin per milliliter.

(4) *Disintegration time.* Proceed as directed in § 436.212 of this chapter, using the procedure described in paragraph (e)(2) of that section.

§ 440.115 Sodium cloxacillin oral dosage forms.

§ 440.115a Sodium cloxacillin capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium cloxacillin capsules are composed of sodium cloxacillin and one or more suitable and harmless diluents and lubricants. Each capsule contains sodium cloxacillin equivalent to 125 milligrams, 250 milligrams, or 500 milligrams of cloxacillin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cloxacillin that it is represented to contain. Its moisture content is not more than 5 percent. The sodium cloxacillin used conforms to the standards prescribed by § 440.15.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The sodium cloxacillin used in making the batch for potency, safety, moisture, pH, sodium cloxacillin content, identity, and crystallinity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The sodium cloxacillin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Place a representative number of capsules into a high-speed glass blender jar containing sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes.

(ii) *Assay procedure.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 5 micrograms of cloxacillin per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this subchapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(2) *Moisture.* Proceed as directed in § 436.201 of this subchapter.

§ 440.115b Sodium cloxacillin for oral solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium cloxacillin for oral solution is a mixture of sodium cloxacillin with one or more suitable and harmless colorings, flavorings, buffer substances, and preservatives. When reconstituted as directed in the labeling, each milliliter contains sodium cloxacillin equivalent to 25 milligrams or 50 milligrams of cloxacillin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cloxacillin that it is represented to contain. Its moisture content is not more than 1 percent. When reconstituted as directed in its labeling, its pH is not less than 5.0 nor more than 7.5. The sodium cloxacillin used conforms to the standards prescribed by § 440.15(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The sodium cloxacillin used in making the batch for potency, safety, moisture, pH, sodium cloxacillin content, identity, and crystallinity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The sodium cloxacillin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Reconstitute the sample as directed in the labeling. Dilute an accurately measured representative aliquot of the sample with sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration.

(ii) *Assay procedures.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 5 micrograms of cloxacillin per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(2) *Moisture.* Proceed as directed in § 436.201 of this subchapter.

(3) *pH.* Proceed as directed in § 436.202 of this subchapter, using the drug reconstituted as directed in its labeling.

§ 440.119 Sodium dicloxacillin monohydrate oral dosage forms.

§ 440.119a Sodium dicloxacillin monohydrate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium dicloxacillin monohydrate capsules are composed of sodium dicloxacillin monohydrate and one or more suitable diluents and lubricants. Each capsule contains sodium dicloxacillin monohydrate equivalent to 62.5, 125, 250, or 500 milligrams of dicloxacillin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of dicloxacillin that it is represented to contain. The moisture content is not more than 5 percent. The sodium dicloxacillin monohydrate conforms to the requirements of § 440.19(a)(1).

$$\text{Percent free chloride} = \frac{N \times \text{milliliters of silver nitrate} \times 3545.7}{\text{Milligrams of sample}}$$

$$\text{Percent total chlorine} = \frac{N \times \text{milliliters of silver nitrate} \times 3545.7}{\text{Milligrams of sample}}$$

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The sodium dicloxacillin monohydrate used in making the batch for potency, safety, moisture, pH, organic chlorine content, free chloride content, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The sodium dicloxacillin monohydrate used in making the batch: 10 containers, each containing not less than 500 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Place a representative number of capsules into a high-speed glass blender jar containing sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 1 to the reference concentration of 5.0 micrograms of dicloxacillin per milliliter (estimated) for the microbiological agar diffusion assay and to the prescribed concentration for the iodometric assay.

(ii) *Assay procedure.* Assay for potency by either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter.

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 440.119b Sodium dicloxacillin monohydrate for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium dicloxacillin monohydrate for oral suspension is a mixture of sodium dicloxacillin monohydrate with one or more suitable colorings, flavorings, buffer substances, and preservatives. When reconstituted as directed in the labeling, it contains the equivalent of 12.5 or 25 milligrams of dicloxacillin per milliliter. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of dicloxacillin that it is represented to contain. Its moisture content is not more than 2 percent. The pH of the suspension, when reconstituted as directed in the labeling, is not less than 4.5 nor more than 7.5. The sodium dicloxacillin monohydrate used conforms to the requirements of § 440.19 (a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assay on:

(*a*) The sodium dicloxacillin monohydrate used in making the batch for potency, safety, moisture, pH, organic chlorine content, free chloride content, crystallinity, and identity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The sodium dicloxacillin monohydrate used in making the batch: 10 containers, each containing not less than 500 milligrams.

(*b*) The batch: A minimum of 6 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Reconstitute the sample as directed in the labeling. Place an accurately measured aliquot of the sample containing an estimated 125 milligrams of dicloxacillin into a 100-milliliter volumetric flask. Add 20 milliliters of dimethylformamide and shake mechanically for 30 minutes. Dilute to volume with 1 percent potassium phosphate buffer, pH 6.0 (solution 1). The addition of dimethylformamide may be omitted if complete solution can be obtained with solution 1. Further dilute an aliquot with sufficient solution 1 to the reference concentration of 5.0 micrograms of dicloxacillin per milliliter (estimated) for the microbiological agar diffusion assay and to the prescribed concentration for the iodometric assay.

(ii) *Assay procedure.* Assay for potency by either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter.

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

§ 440.125 Hetacillin oral dosage forms.

§ 440.125a Hetacillin chewable tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Each hetacillin chewable tablet contains an amount of hetacillin equivalent to 112.5 milligrams of ampicillin with suitable buffers, preservatives, binders, flavorings, colorings, and sweetening ingredients. Its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of ampicillin that it is represented to contain. The moisture content is not more than 2.0 percent. The hetacillin used conforms to the requirements of § 440.25(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The hetacillin used in making the batch for potency, safety, moisture, pH, hetacillin content, identity, and crystallinity.

(*b*) The batch for potency and moisture.

(ii) Samples required.

(*a*) The hetacillin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed for ampicillin in § 436.105 of this chapter, using the ampicillin working standard as the standard of comparison and preparing the sample for assay as follows: Place a representative number of tablets in a high-speed glass blender with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Further dilute an aliquot of the stock solution with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 440.125b Hetacillin for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Hetacillin for oral suspension is a mixture of hetacillin with one or more suitable preservatives, suspending agents, sweetening ingredients, flavorings and colorings. When reconstituted as directed in the labeling, it contains the equivalent of 22.5, 45, or 112.5 milligrams of ampicillin per milliliter. Its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of ampicillin that it is represented to contain. Its moisture content is not more than 2.0 percent. The pH of the suspension, when reconstituted as directed in its labeling, is not less than 2.0 and not more than 5.0. The hetacillin used conforms to the requirements of § 440.25(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The hetacillin used in making the batch for potency, safety, moisture, pH, hetacillin content, identity, and crystallinity.

(*b*) The batch for potency, moisture and pH.

(ii) Samples required:

(*a*) The hetacillin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed for ampicillin in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Remove an accurately measured representative portion with a suitable syringe and hypodermic needle and place into a suitable volumetric flask. Dilute to volume with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3). Further dilute an aliquot with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the sample after reconstituting as directed in the labeling.

§ 440.129 Potassium hetacillin capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Potassium hetacillin capsules are composed of potassium hetacillin with or without one or more suitable diluents, lubricants, and drying agents. Each capsule contains an amount of potassium hetacillin equivalent to 112.5, 225, or 450 milligrams of ampicillin. Its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of ampicillin that it is represented to contain. The moisture content is not more than 3 percent. The potassium hetacillin used conforms to the requirements of § 440.29(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples. In addition to complying with the* requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The potassium hetacillin used in making the batch for potency, safety, moisture, pH, potassium hetacillin content, identity, and crystallinity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The potassium hetacillin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed for ampicillin in § 436.105 of this chapter, using the ampicillin working standard as the standard of comparison and preparing the sample for assay as follows: Place a representative number of capsules in a high-speed gless blender with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Further dilute an aliquot of the stock solution with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 440.141 Sodium nafcillin monohydrate oral dosage forms.

§ 440.141a Sodium nafcillin monohydrate tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium nafcillin monohydrate tablets are composed of nafcillin sodium monohydrate with one or more suitable buffers, binders, disintegrants, diluents, and lubricants. Each tablet contains nafcillin sodium monohydrate equivalent to 500 milligrams of nafcillin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of nafcillin that it is represented to contain. Its moisture content is not more than 5 percent. It shall disintegrate within 20 minutes. The nafcillin sodium monohydrate used conforms to the standards prescribed by § 440.41(a)(1).

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5 of this chapter, this drug shall be labeled "nafcillin sodium tablets".

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nafcillin sodium monohydrate used in making the batch for potency, safety, moisture, pH, crystallinity, nafcillin sodium monohydrate content, and identity.

(*b*) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(*a*) The nafcillin sodium monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Place a representative number of tablets into a high-speed glass blender jar containing sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes.

(ii) *Assay procedures.* Assay for potency by any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 2.0 micrograms of nafcillin per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(*c*) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter, using the method described in paragraph (e)(1) of that section, except use distilled water in lieu of simulated gastric fluid as the immersion fluid.

§ 440.141b Sodium nafcillin monohydrate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium nafcillin monohydrate capsules are composed of sodium nafcillin monohydrate and one or more suitable and harmless buffer substances and lubricants. Each capsule contains sodium nafcillin monohydrate equivalent to 250 milligrams of nafcillin. The potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of nafcillin that it is represented to contain. The moisture content is not more than 5.0 percent. The sodium nafcillin monohydrate conforms to the standards prescribed by § 440.41(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The sodium nafcillin monohydrate used in making the batch for potency, safety, moisture, pH, crystallinity, sodium nafcillin monohydrate content, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The sodium nafcillin monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Place a representative number of capsules into a high-speed glass blender jar containing sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 1 to the reference concentration of 2.0 micrograms of nafcillin per milliliter (estimated) for the microbiological agar diffusion assay and to the prescribed concentration for the iodometric assay.

(ii) *Assay procedures.* Assay for potency by either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter.

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 440.141c Sodium nafcillin monohydrate for oral solution.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Sodium nafcillin monohydrate for oral solution is a packaged combination of one immediate container of sodium nafcillin monohydrate and one immediate container of an aqueous diluent containing one or more suitable and harmless colorings, flavoring, buffers, dispersants, diluents, and preservatives. When reconstituted as directed in the labeling, each milliliter contains sodium nafcillin monohydrate equivalent to 50 milligrams of nafcillin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of nafcillin that it is represented to contain. Its moisture content is not more than 5 percent. When reconstituted as directed in the labeling, its pH is not less than 5.5 and not more than 7.5. The sodium nafcillin monohydrate used conforms to the standards prescribed by § 440.41(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The sodium nafcillin monohydrate used in making the batch for potency, safety, moisture, pH, crystallinity, sodium nafcillin monohydrate content, and identity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The sodium nafcillin monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 6 immediate containers.

(b) *Tests and methods of assay—*(1) *Potency—*(i) *Sample preparation.* Reconstitute as directed in the labeling. Place an accurately measured representative aliquot of the sample into a 250-milliliter volumetric flask and dilute to volume with 1 percent potassium phosphate buffer, pH 6.0 (solution 1). Mix well. Further dilute an aliquot with solution 1 to the reference concentration of 2.0 micrograms of nafcillin per milliliter (estimated) for the microbiological agar diffusion assay and to the prescribed concentration for the iodometric assay.

(ii) *Assay procedures.* Assay for potency by either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter.

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

§ 440.149 Sodium oxacillin oral dosage forms.

§ 440.149a Sodium oxacillin capsules.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Sodium oxacillin capsules are composed of sodium oxacillin with or without one or more diluents and lubricants, enclosed in a gelatin capsule. Each capsule contains sodium oxacillin equivalent to 125, 250, or 500 milligrams of oxacillin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of oxacillin that it is represented to contain. Its moisture content is not more than 6.0 percent. The sodium oxacillin used conforms to the standards prescribed by § 440.49(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The sodium oxacillin used in making the batch for potency, safety, moisture, pH, sodium oxacillin content, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The sodium oxacillin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay—*(1) *Potency—*(i) *Sample preparation.* Place a representative number of capsules into a high-speed glass blender jar containing sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes.

(ii) *Assay procedures.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 5 micrograms of oxacillin per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 440.149b Sodium oxacillin for oral solution.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Sodium oxacillin for oral solution is a mixture of sodium oxacillin with one or more suitable colorings, flavorings, buffer substances, stabilizers, and preservatives. When reconstituted as directed in the labeling, each milliliter contains sodium oxacillin equivalent to either 25 or 50 milligrams of oxacillin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of oxacillin that it is represented to contain. Its moisture content is not more than 1.0 percent. When reconstituted as directed in its labeling, the pH of the solution is not less than 5.0 and not more than 7.5. The sodium oxacillin used conforms to the standards prescribed by § 440.49(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Request for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The sodium oxacillin used in making the batch for potency, safety, moisture, pH, sodium oxacillin content, crystallinity, and identity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The sodium oxacillin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay—*(1) *potency—*(i) *Sample preparation.* Reconstitute as directed in the labeling. Place an accurately measured representative aliquot of the sample into an appropriate-sized volumetric flask with sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration.

(ii) *Assay procedures.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 5 micrograms of oxacillin per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter using the drug reconstituted as directed in the labeling.

§ 440.151 Penicillamine capsules.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Penicillamine capsules each contain 250 milligrams of penicillamine and one or more suitable and harmless diluents and lubricants. Its content of penicillamine is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of penicillamine that it is represented to contain. The moisture content is not more than 7.5 percent. Each cap-

sule contains not more than 0.1 unit of penicillin activity. The penicillamine used conforms to the requirements of § 440.51(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The penicillamine used in making the batch for penicillamine content, safety, loss on drying, pH, penicillin activity, residue on ignition, heavy metals, specific rotation, and identity.

(*b*) The batch for penicillamine content, moisture, and penicillin activity.

(ii) Samples required:

(*a*) The penicillamine used in making the batch: 10 packages, each containing approximately 1 gram.

(*b*) The batch: A minimum of 40 capsules.

(b) *Tests and methods of assay*—(1) *Penicillamine content.* Proceed as directed in § 440.51(b)(1), except prepare the sample for assay as follows: Empty the contents of not less than 10 capsules into a tared weighing bottle. Weigh and mix the powder. Calculate the average capsule weight content and dissolve an accurately weighed quantity of the mixed capsule powder equivalent to about 300 milligrams of penicillamine in 200 milliliters of water. Calculate the penicillamine content for the sample used and determine the penicillamine content for the average capsule weight.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Penicillin activity.* Proceed as directed in § 436.104 of this chapter, except use the contents of 2 capsules in lieu of 1.0 gram of the sample.

§ 440.153 Aluminum penicillin tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Aluminum penicillin tablets are tablets composed of aluminum penicillin and sodium benzoate, with or without one or more suitable and harmless diluents, binders, lubricants, colorings, and flavorings. The potency of each tablet is not less than 50,000 units, and if it is less than 100,000 units it is "unscored". Each tablet contains 0.3 gram of sodium benzoate. Its moisture content is not more than 2 percent. Tablets not exceeding 15 millimeters in diameter, or not intended only for preparing solutions, shall disintegrate within 1 hour. The aluminum penicillin used conforms to the requirements of § 440.53a(a)(1) for aluminum penicillin except paragraph (a)(1) (ii) and (iii) thereof. Each other substance used in the preparation of aluminum penicillin tablets, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* Unless each aluminum penicillin tablet is enclosed in foil or plastic film and such enclosure is a tight container as defined by the U.S.P., except the provision that it shall be capable of tight reclosure, the immediate container shall be a tight container as so defined. The immediate container may also contain a desiccant separated from the tablets by a plug of cotton or other like material. The composition of the immediate container, or of the foil or film enclosure, shall be such as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded. The number of tablets in the immediate container is such that the total number of units therein is not less than 300,000.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the outside wrapper or container and the immediate container, the statement "Expiration date _____", the blank being filled in with the date that is 24 months after the month during which the batch was certified.

(ii) On the outside wrapper or container, the statement "Store in refrigerator not above 15° C. (59° F.)" or "Store below 15° C. (59° F.)".

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of tablets in such batch, the number of tablets of the batch packaged into dispensing-size containers during each day's packaging operations, the number of units in each tablet, the date on which the latest assay of the drug comprising such batch was completed, the batch mark and date (unless submitted previously) on which the latest assay of the penicillin used in making such batch was completed, the quantity of each ingredient used in making the batch, and a statement that each such ingredient conforms to the requirements prescribed therefor, if any, by this section.

(ii) Except as otherwise provided in paragraph (a)(4)(iv) of this section, such person shall submit in connection with his requests results of the tests and assays listed after each of the following made by him on an accurately representative sample of:

(*a*) The batch:

(*1*) If the person who requests certification is the manufacturer of the batch: Average potency, average moisture, and, if required by paragraph (a)(1) of this section, disintegration time of tablets collected during the time of tableting the batch; and, unless the tablets are packaged into dispensing-size containers immediately after they are compressed, average moisture of tablets collected during each day of packaging the batch.

(*2*) If the person who requests certification is not the manufacturer of the batch: Average potency, average moisture, and, if required by paragraph (a)(1) of this section, disintegration time of tablets collected during each day the tablets are being packaged into dispensing-size containers.

(*b*) The aluminium penicillin used in making the batch; potency, moisture, pH, toxicity, and penicillin K content.

(iii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch:

(*1*) For potency and moisture:

(*i*) If the person who requests certification is the manufacturer of the batch: One tablet for each 5,000 tablets in the batch, but in no case less than 30 tablets collected by taking single tablets at such intervals throughout the entire time of tableting the batch that the quantities tableted during the intervals are approximately equal;

(*ii*) If, after tableting, such person packages the batch into dispensing-size containers: 20 tablets collected at equal intervals during each day the tablets are being packaged, except that this sample is not required if the tablets are packaged immediately after they are compressed; or

(*iii*) If the person who requests certification is not the manufacturer of the batch (for the pruposes of certification, a batch shall be that number of tablets filled by such person into dispensing-size containers during each day's packaging operations): One tablet for each 5,000 tablets in the batch, but in no case less than 30 tablets collected by taking single tablets at such intervals throughout each day of packaging the tablets that the quantities packaged during the intervals are approximately equal.

(*2*) For disintegration time: 6 tablets.

(*b*) The aluminum penicillin used in making the batch; 6 packages, each containing approximately equal portions of not less than 300 milligrams each, packaged in accordance with the requirements of § 440.53a(a)(2).

(*c*) In case of an initial request for certification, each other substance used in making the batch; one package of each containing approximately five grams.

(iv) No result referred to in paragraph (a)(4)(ii)(*b*) of this section, and no sample referred to in paragraph (a)(4)(iii)(*b*) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 440.180a(b)(1), using citrate buffer at pH 6.3 for making working dilutions of the working standards and for the sample under test in lieu of phosphate buffer. The citrate buffer is of the following composition:

	Grams
Citric acid	52.9
Sodium hydroxide (pellets)	28.25
Sodium citrate	388.0

Make up to 4000 ml. with distilled water.

(2) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i).

(3) *Disintegration time.* Proceed as directed in § 440.180a(b)(3).

§ 440.155 Benzathine penicillin G oral dosage forms.

§ 440.155a Benzathine penicillin G and and crystalline penicillin tablets.

(a) *Requirements for certification.* Benzathine penicillin G and crystalline penicillin tablets conform to all requirements prescribed therefor by § 440.180a(a) for penicillin tablets and are subject to all procedures prescribed by that section for penicillin tablets, except that:

(1) Each tablet contains not less than 100,000 units of benzathine penicillin G and not less than 100,000 units of crystalline penicillin sodium or potassium penicillin.

(2) Their moisture content is not more than 4 percent.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Total potency.* Proceed as directed in § 440.180a(b)(1)(ii). Its total potency is satisfactory if it contains not less than 85 percent of the number of units per tablet that it is represented to contain.

(ii) *Crystalline penicillin content.* Place 5 tablets in a blending jar and add thereto sufficient distilled water to give a concentration of 5,000 units of crystalline penicillin per milliliter. Blend for 2 minutes with a high-speed blender, filter, remove a 10-milliliter aliquot of the filtrate and dilute to 25 milliliters with 1.0 percent phosphate buffer at pH 6.0. Remove a 2-milliliter aliquot of this solution and assay by the iodometric assay procedure described in § 440.80a(b)(5)(iv)(*a*). Its content of crystalline penicillin is satisfactory if it contains not less than 85 percent of the number of units per tablet that it is represented to contain.

(iii) *Benzathine penicillin G content.* The benzathine penicillin G content of the batch is the difference between the total potency as determined by the method described in paragraph (b)(1)(i) of this section and the content of crystalline penicillin as determined by the method prescribed in paragraph (b)(1)(ii) of this section. Its content of benzathine penicillin G is satisfactory if it contains not less than 85 percent of the number of units per tablet that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 440.74a(b)(5).

(c) *Disintegration time.* Proceed as directed in § 440.180a(b)(3).

§ 440.155b Benzathine penicillin G and phenoxymethyl penicillin tablets.

(a) *Requirements for certification.* (1) Benzathine penicillin G and phenoxymethyl penicillin tablets conform to all requirements and are subject to all procedures prescribed by § 440.180a(a) for tablets benzathine penicillin G, except that each tablet contains not less than 50,000 units of phenoxymethyl penicillin.

(2) In addition to complying with the requirements of § 440.180a(a)(4), a person who requests certification of a batch shall submit with his request a statement showing the batch mark and (unless they were previously submitted) the results and the date of the latest tests and assays of the phenoxymethyl penicillin used in making the batch for potency, toxicity, moisture, pH, crystallinity, and phenoxymethyl penicillin content. He shall also submit in connection with his request (unless it was previously submitted) a sample consisting of 10 packages containing approximately equal portions of not less than 300 milligrams each of the phenoxymethyl penicillin used in making the batch, packaged in accordance with the requirements of § 440.71(a)(2).

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Total penicillin content.* Accurately weigh 6 tablets and grind them to a fine powder. Assay this powder by the iodometric method described in § 440.80a(b)(5)(iv)(*a*), except in preparing the blank solution accurately weigh a portion of the powder equivalent to approximately 200,000 units of penicillin and make a suspension in 1-percent phosphate buffer, pH 6.0, with a volume of 100 milliliters. Shake well, pipette 2.0 milliliters into a 125-milliliter glass-stoppered Erlenmeyer flask, add 10.0 milliliters of 0.01*N* iodine and immediately titrate with 0.01*N* $Na_2S_2O_3$. In preparing the solution for inactivation, accurately weigh a portion of the powder equivalent to 200,000 units of penicillin and place in a 100-milliliter volumetric flask. Add 20 milliliters of 0.5*N* NaOH and mix well. Allow to stand for 15 minutes, then add 10 milliliters of 1.2 *N* HCl, and make to volume with distilled water. Pipette a 2.0-milliliter aliquot into a 125-milliliter glass-stoppered Erlenmeyer flask and add 10.0 milliliters of 0.01*N* iodine. Allow to stand for 15 minutes and then titrate the excess iodine with 0.01*N* $Na_2S_2O_3$. Penicillin G is used as the standard of comparison. The total penicillin content is satisfactory if it is not less than 85 percent of that which it is represented to contain.

(ii) *Phenoxymethyl penicillin content.* Place in a 125-milliliter separatory funnel an accurately weighed quantity of finely ground tablet powder equivalent to approximately 20 milligrams (33,900 units) of phenoxymethyl penicillin. Add 50.0 milliliters of spectrophotometric grade chloroform and 1 milliliter of 1:4 H_3PO_4. Shake well for 2 minutes. Allow the layers to separate, filter the lower chloroform layer through a cotton pledget, and collect the clear chloroform solution. Determine the absorbance of the chloroform solution at the absorption peak at 276 mμ, using a suitable ultraviolet spectrophotometer and 1-centimeter quartz cells. Set the instrument to 100-percent transmission with chloroform. If a recording spectrophotometer is used, record the ultraviolet absorption spectrum from 250–290mμ. If a nonrecording spectrophotometer is used, the original sample used should be equivalent to approximately 10 milligrams of phenoxymethyl penicillin. Determine the absorbance of the solution at the 276 mμ absorption peak. (The exact position of the peak should be determined for the particular instrument used.) Calculate the amount of phenoxymethyl penicillin per tablet as follows:

$$\text{Phenoxymethyl penicillin units per tablet} = \frac{\text{Absorbance at 276 m}\mu \times \text{average weight of each tablet} \times 1{,}695{,}000}{\text{Weight of powder taken} \times 2 \times a_{vm}}$$

where:

a_{vm} = absorptivity (1%, 1 cm.) of phenoxymethyl penicillin working standard in chloroform, similarly treated.

The phenoxymethyl penicillin content is satisfactory if it is not less than 85 percent of that which it is represented to contain.

(iii) *Penicillin G content.* The penicillin G content of the batch is the difference between the total penicillins determined by the method described in paragraph (b)(1)(i) of this section and the phenoxymethyl penicillin content determined by the method described in paragraph (b)(1)(ii) of this section. The penicillin G content is satisfactory if it is not less than 85 percent of that which it is represented to contain.

(2) *Moisture.* Proceed as directed in § 440.74a(b)(5).

(3) *Disintegration time.* Proceed as directed in § 440.180a(b)(3).

§ 440.155c Benzathine penicillin G oral suspension, benzathine penicillin G for oral suspension (benzathine penicillin G powder).

(a) *Requirements for certification.* (1) *Standards of identity, strength, quality, and purity.* Benzathine penicillin G oral suspension is an aqueous suspension of benzathine penicillin G, and benzathine penicillin G for oral suspension is a dry mixture of benzathine penicillin G; each drug may contain one or more suitable and harmless dispersing agents, buffer substances, and preservatives, and suitable and harmless colorings and flavorings. The potency of the oral suspension and of the suspension prepared as directed in its labeling is not less than 20,000 units per milliliter. Its pH is not less than 6.0 and not more than 7.0. If it is a dry mixture of the drug, its moisture content is not more than 6.0 percent. The benzathine penicillin G used conforms to the requirements of § 440.55a(a)(1), except paragraph (a)(1)(ii), (iv), and, if it is the oral suspension, (v) of that section. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* The immediate container shall be a tight container as defined by the U. S. P. and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage,

and distribution practice shall be disregarded.

(3) *Labeling.* In addition to the requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the outside wrapper or container and the immediate container the statement "Expiration date _____", the blank being filled in with the date that is 24 months after the month during which the batch was certified, except that if it is the dry mixture of the drug the blank may be filled in with the date that is 36 months after the month during which the batch was certified, if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time such drug as prepared by him conforms with the standards prescribed by paragraph (a)(1) of this section.

(ii) [Reserved]

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless it was previously submitted) the date on which the latest assay of the benzathine penicillin G used in making such batch was completed, the potency per milliliter if it is a suspension of the drug and the number of units in each immediate container if it is a dry mixture, the date on which the latest assay of the drug comprising such batch was completed, the quantity of each ingredient used in making the batch, and a statement that each such ingredient conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided in paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request results of the tests and assays listed after each of the following, made by him on an accurately representative sample of:

(*a*) The batch; average potency per milliliter if it is the oral suspension, average potency per immediate container if it is a dry mixture, pH, and moisture if it is a dry mixture of the drug.

(*b*) The benzathine penicillin G used in making the batch; potency, toxicity, pH, moisture (if it is used in the preparation of a dry mixture), crystallinity, and the penicillin G content.

(iii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch; one immediate container for each 5,000 immediate containers in the batch, but in no case less than 5 immediate containers, collected by taking single immediate containers at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(*b*) The benzathine penicillin G used in making the batch; 10 packages, each containing approximately equal portions of not less than 300 milligrams, packaged in accordance with the requirements of § 440.55a(a)(2).

(*c*) In case of an initial request for certification, each other ingredient used in making the batch; one package of each containing approximately 5 grams.

(iv) No result referred to in paragraph (a)(4)(ii)(*b*) of this section, and no sample referred to in paragraph (a)(4)(iii)(*b*) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 440.55a(b)(1), using as the sample for assay a representative aliquot of the oral suspension; or if it is a dry mixture of the drug, a representative aliquot of the drug, reconstituted as directed in the labeling. Its potency is satisfactory if it contains not less than 85 percent of the number of units that it is represented to contain.

(2) *pH.* Proceed as directed in § 440.80a(b)(5)(ii), using the undiluted aqueous suspension or the suspension prepared as directed in the labeling of the drug.

(3) *Moisture (if it is a dry mixture of the drug).* Proceed as directed in § 440.74a(b)(5).

§ 440.157 Benzathine phenoxymethyl penicillin oral suspension; benzathine phenoxymethyl penicillin for oral suspension.

(a) *Requirements for certification.* Benzathine phenoxymethyl penicillin oral suspension and benzathine phenoxymethyl penicillin for oral suspension conform to all requirements and are subject to all procedures prescribed by § 440.155c(a) for benzathine penicillin G oral suspension and benzathine penicillin G for oral suspension, except that:

(1) If it is benzathine phenoxymethyl penicillin for oral suspension, its pH is not less than 5.0 and not more than 7.0.

(2) Benzathine phenoxymethyl penicillin is used in lieu of benzathine penicillin G. The benzathine phenoxymethyl penicillin used conforms to the requirements of § 440.57a(a)(1).

(3) In lieu of the directions prescribed by § 440.155c(a)(4)(ii)(*b*), a person who requests certification of a batch shall submit in connection with his request results of the tests and assays made by him on an accurately representative sample of the benzathine phenoxymethyl penicillin used in making the batch for potency, toxicity, pH, moisture, crystallinity, and the phenoxymethyl penicillin content.

(b) *Tests and methods of assay*—(1) *Potency.* Use as the sample for assay a representative aliquot of the oral suspension; or if it is a dry mixture of the drug, use a representative aliquot of the drug reconstituted as directed in the labeling and proceed as directed in § 440.55a(b)(1), except use the phenoxymethyl penicillin working standard as the standard of comparison. Its potency is satisfactory if it contains not less than 85 percent of the number of units that it is represented to contain.

(2) *pH.* Proceed as directed in § 440.80a(b)(5)(ii), using the undiluted aqueous suspension or the suspension prepared as directed in the labeling of the drug.

(c) *Moisture (if it is the dry mixture of the drug).* Proceed as directed in § 440.74a(b)(5).

§ 440.160 Dibenzylamine penicillin and potassium penicillin powder, buffered.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Dibenzylamine penicillin and potassium penicillin powder, buffered, is a dry mixture of dibenzylamine penicillin G and potassium penicillin G, and with one or more suitable and harmless buffer substances, colorings, and flavorings. It shall contain not less than 100,000 units of potassium penicillin G for each 200,000 units of dibenzylamine penicillin G. Its moisture content is not more than 1 percent. Its pH is not less than 5.5 and not more than 7.5. The dibenzylamine penicillin G used conforms to the requirements of § 440.60a(a)(1), except paragraph (a)(1)(ii) and (iv) of that section. The potassium penicillin used conforms to the requirements of § 440.80a(a)(1) for potassium penicillin, except paragraph (a)(1)(ii) and (iv) of that section. Each other ingredient used, if its name is recognized in the U.S.P. or N.F. conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* In all cases the immediate container shall be a tight container as defined by the U.S.P. The composition of the immediate container shall be such as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused that are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the outside wrapper or container and the immediate container the statement "Expiration date _____", the blank being filled in with the date that is 12 months after the month during which the batch was certified.

(ii) [Reserved]

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless they were previously submitted) the dates on

which the latest assays of the dibenzylamine penicillin G and the potassium penicillin G used in making the batch were completed, the number of units of dibenzylamine penicillin G and the number of units of potassium penicillin G in each container of the batch, the date on which the latest assay comprising such batch was completed, the quantity of each ingredient used in making the batch, and a statement that each such ingredient conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided in paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request results of the tests and assays listed after each of the following, made by him on an accurately representative sample of:

(*a*) The batch; potency and moisture.

(*b*) The dibenzylamine penicillin G used in making the batch; potency, toxicity, moisture, pH, crystallinity, and penicillin G content.

(*c*) The potassium penicillin G used in making the batch; potency, toxicity, moisture, pH, penicillin G content, crystallinity, and heat stability.

(iii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch; 1 immediate container for each 5,000 immediate containers in the batch, but in no case less than 6 immediate containers, collected by taking single immediate containers at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(*b*) The dibenzylamine penicillin G used in making the batch; 3 packages, each containing approximately equal portions of not less than 500 milligrams packaged in accordance with the requirements of § 440.60a(a)(2).

(*c*) The potassium penicillin G used in making the batch; 3 packages, each containing approximately equal portions of not less than 250 milligrams packaged in accordance with the requirements of § 440.80a(a)(2).

(*d*) In case of an initial request for certification, each other ingredient used in making the batch; 1 package of each containing approximately 5 grams.

(iv) No result referred to in paragraph (a)(4)(ii) (*b*) and (*c*) of this section, and no sample referred to in paragraph (a)(4)(iii) (*b*) and (*c*) of this section, is required if such results or samples have been previously submitted.

(b) *Tests and methods of assay*—(1) *Total potency.* Proceed as directed in § 440.80a(b)(1), using 5 milliliters of the preparation, reconstituted as directed in the labeling.

(2) *Potassium penicillin content.* Centrifuge approximately 10 milliliters of the reconstituted preparation to obtain a clear solution and proceed as directed in § 440.80a(b)(1), using 5 milliliters of the clear solution. The potency of the clear solution is regarded as the potassium penicillin content. The content of potassium penicillin is satisfactory if it is not less than 85 percent of that which it is represented to contain.

(3) *Dibenzylamine penicillin content.* The difference between the total potency as determined by paragraph (b)(1) of this section and the potassium penicillin as determined by paragraph (b)(2) of this section represents the amount of dibenzylamine penicillin present. The content of dibenzylamine penicillin is satisfactory if it is not less than 85 percent of that which it is represented to contain.

(4) *Moisture.* Proceed as directed in § 440.74a(b)(5).

(5) *pH.* Proceed as directed in § 440.80a(b)(5)(ii), using the suspension resulting when the product is reconstituted as directed in the labeling.

§ 440.166 Hydrabamine penicillin G oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Hydrabamine penicillin G oral suspension is hydrabamine penicillin G and one or more suitable and harmless suspending or dispersing agents, buffer substances, and preservatives, with or without one or more suitable and harmless colorings and flavorings. Its potency is not less than 60,000 units per milliliter. Its pH is not less than 5.5 and not more than 7.0. The hydrabamine penicillin G used conforms to the requirements of § 440.66(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* The immediate container shall be a tight container as defined by the U.S.P. and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused that are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its outside wrapper or container and the immediate container, as hereinafter indicated, the following:

(i) The statement "Shake well."

(ii) The statement "Expiration date ----------", the blank being filled in with the date that is 12 months after the month during which the batch was certified.

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless it was previously submitted) the date on which the latest assay of the hydrabamine penicillin G used in making such batch was completed, the potency per milliliter of the batch, the date on which the latest assay of the drug comprising such batch was completed, the quantity of each ingredient used in making the batch, and a statement that each such ingredient conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided in paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request results of the tests and assays listed after each of the following, made by him on an accurately representative sample of:

(*a*) The batch: average potency per milliliter, pH.

(*b*) The hydrabamine penicillin G used in making the batch: potency, toxicity, moisture, pH, crystallinity, the penicillin G content, and the extinction coefficient.

(iii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch: 1 immediate container for each 5,000 immediate containers in the batch, but in no case less than 5 immediate containers, collected by taking single immediate containers at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(*b*) The hydrabamine penicillin G used in making the batch: 10 packages, each containing equal portions of not less than 300 milligrams, packaged in accordance with the requirements of § 440.66(a)(2).

(*c*) In case of an initial request for certification, each other ingredient used in making the batch; one package of each containing approximately 5 grams.

(iv) No result referred to in paragraph (a)(4)(ii)(*b*) of this section, and no sample referred to in paragraph (a)(4)(iii)(*b*) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 440.80a(b)(1), except if the bioassay method is § 141a.1, except if the bioassay method is used prepare the sample as follows: Place 1.0 milliliter of the sample to be tested in a 100-milliliter volumetric flask and dilute to volume with methanol. By means of a volumetric pipette add a 1.0-milliliter aliquot of this solution to a sufficient volume of 1-percent phosphate buffer pH 6.0 to give a solution having a concentration of 1 unit per milliliter (constantly swirling the flask during the addition). If the iodometric method is used, dilute 1.0 milliliter of the sample with sufficient 1-percent phosphate buffer pH 6.0 to produce a suspension containing 2,000 units per milliliter (estimated). Mix and pipette 2.0 milliliters into a 125-milliliter glass-stoppered Erlenmeyer flask. Add 10 milliliters of 0.01*N* I_2 and titrate immediately with 0.01*N* $Na_2S_2O_3$ for the blank determination. Dilute a second 1.0-milliliter portion of the sample with 1*N* NaOH to

produce a suspension containing 2,000 units per milliliter (estimated) and add approximately half that amount of chloroform U.S.P. Shake immediately and again after 5 minutes. Fifteen minutes after the initial shaking, pipette 2.0 milliliters of the upper NaOH layer into a 125-milliliter glass-stoppered Erlenmeyer flask and add 2.0 milliliters of 1.2*N* HCl and 10 milliliters of 0.01*N* I_2. After 15 minutes titrate the excess iodine with 0.01*N* $Na_2S_2O_3$.

$$\text{Units of penicillin per milliliter of suspension} = \frac{\text{Difference in titers} \times \text{potency of F.D.A. working standard in units per milligram} \times \text{volume of 2,000 units per milliliter suspension}}{F \times 2}$$

where F = the number of milliliters of 0.01*N* I_2 absorbed by 1.0 milligram of the Food and Drug Administration sodium penicillin G working standard.

(2) *pH.* Proceed as directed in § 440.80a(b)(5)(ii), using the undiluted aqueous suspension.

§ 440.169 Hydrabamine phenoxymethyl penicillin oral dosage forms.

§ 440.169a Hydrabamine phenoxymethyl penicillin chewable tablets.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Hydrabamine phenoxymethyl penicillin chewable tablets are composed of hydrabamine phenoxymethyl penicillin with suitable diluents, binders, buffers, colorings, and flavorings. Each tablet contains hydrabamine phenoxymethyl penicillin equivalent to 125 milligrams (200,000 units) or 250 milligrams (400,000 units) of phenoxymethyl penicillin. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams or units of phenoxymethyl penicillin that it is represented to contain. The loss on drying is not more than 2 percent. The hydrabamine phenoxymethyl penicillin used conforms to the standards prescribed by § 440.69(a)(1).

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5 of this chapter, this drug shall be labeled "hydrabamine phenoxymethyl penicillin tablets."

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The hydrabamine phenoxymethyl penicillin used in making the batch for potency, safety, moisture, pH, hydrabamine absorptivity, phenoxymethyl penicillin content, and crystallinity.

(*b*) The batch for potency and loss on drying.

(ii) Samples required:

(*a*) The hydrabamine phenoxymethyl penicillin used in making the batch: 10 packages, nine containing approximately equal portions of not less than 300 milligrams and one containing not less than 2 grams.

(*b*) The batch: A minimum of 30 tablets.

(b) *Tests and methods of assay—*(1) *Potency.* Using the phenoxymethyl penicillin working standard as the standard of comparison, assay for potency by either of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of tablets into a glass blending jar with sufficient methyl alcohol to give a convenient concentration. Blend for 3 to 5 minutes. Further dilute an aliquot with methyl alcohol to 1,000 units of phenoxymethyl penicillin per milliliter (estimated). By means of a volumetric pipet, add dropwise a 1.0-milliliter aliquot of the 1,000-units-per-milliliter concentration to a 1-liter volumetric flask containing approximately 800 milliliters of 1 percent potassium phosphate buffer, pH 6.0 (solution 1), constantly swirling the flask during the addition. Dilute to volume with solution 1.

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, preparing the sample as follows: Weigh and finely powder 10 tablets. Transfer an accurately weighed portion of the powdered tablets, equivalent to about 200,000 units, to a 100-milliliter volumetric flask. Dissolve the powder in chloroform, dilute to volume with chloroform, and mix. Pipet 10 milliliters of the solution into each of two 50-milliliter glass-stoppered centrifuge tubes. To one tube add 10.0 milliliters of 1*N* sodium hydroxide. To the other tube (which is to be used as the blank) add 10.0 milliliters of 1 percent potassium phosphate buffer, pH 6.0 (solution 1). Shake both tubes vigorously for 20 to 30 seconds, and then centrifuge. Use the buffer layer for the blank determination. In lieu of § 436.204(c)(1) and (2) of this chapter, pipet 2.0 milliliters of the alkaline layer from the first tube into a glass-stoppered Erlenmeyer flask and let stand for a total of 15 minutes from the time the sodium hydroxide was added to the chloroform solution. At the end of the 15-minute waiting period, proceed as directed in § 436.204(c)(3) and (4) of this chapter.

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

§ 440.169b Hydrabamine phenoxymethyl penicillin oral suspension.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Hydrabamine phenoxymethyl penicillin oral suspension is composed of hydrabamine phenoxymethyl penicillin and one or more suitable suspending or dispersing agents, buffer substances, preservatives, colorings, and flavorings. Each milliliter contains hydrabamine phenoxymethyl penicillin equivalent to 36 milligrams (60,000 units) of phenoxymethyl penicillin. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams or units of phenoxymethyl penicillin that it is represented to contain. Its pH is not less than 4.5 and not more than 6.5. The hydrabamine phenoxymethyl penicillin used conforms to the standards prescribed by § 440.69(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the quirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The hydrabamine phenoxymethyl penicillin used in making the batch for potency, safety, moisture, pH, hydrabamine absorptivity, phenoxymethyl penicillin content, and crystallinity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The hydrabamine phenoxymethyl penicillin used in making the batch: 10 packages, nine containing approximately equal portions of not less than 300 milligrams and one containing not less than 2 grams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay—*(1) *Potency.* Using the phenoxymethyl penicillin working standard as the standard of comparison, assay for potency by either of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place 1.0 milliliter of the sample into a 100-milliliter volumetric flask and dilute to volume with methyl alcohol. Further dilute an aliquot with methyl alcohol to give a solution containing 1,000 units of phenoxymethyl penicillin per milliliter (estimated). By means of a volumetric pipet, add dropwise a 1.0-milliliter aliquot of the 1,000-units-per-milliliter concentration to a 1-liter volumetric flask containing approximately 800 milliliters of 1 percent potassium phosphate buffer, pH 6.0 (solution 1), constantly swirling the flask during the addition. Dilute to volume with solution 1.

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, preparing the sample as follows: Dilute 1.0 milliliter of the sample with 1*N* NaOH to obtain a suspension containing 2,000 units per milliliter (estimated) and add approximately half the amount of chloroform U.S.P. Shake well immediately and again after 5 minutes. Allow to stand for a total of 15 minutes. Use the upper NaOH layer as the sample. For the blank determination, dilute 1.0 milliliter of the sample with sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to obtain a suspension containing 2,000 units per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted drug.

§ 440.171 Phenoxymethyl penicillin oral dosage forms.

§ 440.171a Phenoxymethyl penicillin capsules.

(a) *Requirements for certification.* Phenoxymethyl penicillin capsules are capsules that conform to all the requirements and are subject to all procedures prescribed by § 440.180a(a) for phenoxymethyl penicillin tablets except with respect to disintegration time, and except that the moisture content of such capsules is not more than 2 percent.

(b) *Tests and methods of assay*—(1) *Potency.* Using the phenoxymethyl penicillin working standard as the standard of comparison, proceed as directed in § 440.80a(b)(1), except prepare the sample as follows: Dissolve the contents of a representative number of capsules (usually 5 to 12) in sufficient redistilled methanol to give a concentration of not more than 50,000 units per milliliter, and further dilute with sufficient phosphate buffer, pH 6.0, to give a concentration of 2,000 units per milliliter (estimated) if the iodometric chemical assay is used, or 1.0 unit per milliliter (estimated) if the bioassay method is used. The average potency of phenoxymethyl penicillin capsules is satisfactory if it is not less than 85 percent of the number of units per capsule that it is represented to contain.

(2) *Moisture.* Use the contents of 4 capsules and proceed as directed in § 440.74a(b)(5).

§ 440.171b Phenoxymethyl penicillin for oral suspension; potassium phenoxymethyl penicillin for oral solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Phenoxymethyl penicillin for oral suspension or potassium phenoxymethyl penicillin for oral solution is a mixture of phenoxymethyl penicillin or potassium phenoxymethyl penicillin, with or without one or more suitable and harmless suspending agents, coloring, flavorings, buffer substances, and preservatives. Its moisture content is not more than 1 percent. The phenoxymethyl penicillin used conforms to the requirements of § 440.71(a)(1). The potassium phenoxymethyl penicillin used conforms to the requirements of § 440.73(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* In all cases the immediate container shall be a tight container as defined by the U.S.P., and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused that are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded. Each immediate container may be packaged in combination with a container of a suitable and harmless aqueous vehicle.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the outside wrapper or container and the immediate container, the statement "Expiration date _____", the blank being filled in with the date that is 24 months after the month during which the batch was certified, except that if it contains sulfonamides the blank shall be filled in with the date that is 12 months after the month during which the batch was certified, and except that the blank may be filled in with the date that is 24 months after the month in which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time such drug as prepared by him complies with the standards prescribed by paragraph (a)(1) of this section.

(ii) [Reserved]

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless it was previously submitted) the date on which the latest assay of the penicillin used in making such batch was completed, the number of units in each immediate container, the quantity of each ingredient used in making the batch, the date on which the latest assay of the drug comprising such batch was completed, and a statement that each ingredient used in making the batch conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided in paragraph (a)(4)(iv) of this section such person shall submit in connection with his request results of the tests and assays listed after each of the following, made by him on an accurately representative sample of:

(*a*) The batch: Potency and moisture.

(*b*) The phenoxymethyl penicillin or potassium phenoxymethyl penicillin used in making the batch: Potency, toxicity, moisture, pH, crystallinity, and phenoxymethyl penicillin content.

(iii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch: One immediate container for each 5,000 immediate containers in the batch, but in no case less than 5 immediate containers, collected by taking single immediate containers at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(*b*) The penicillin used in making the batch: 10 packages, each containing approximately equal portions of not less than 300 milligrams, packaged in accordance with the requirements of § 440.71(a)(2).

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each, containing approximately 5 grams.

(*d*) In case of an initial request for certification of a batch that is to be packaged in combination with an aqueous vehicle, or when any change is made in the composition of such aqueous vehicle: Five packages of the vehicle included in the combination.

(iv) No result referred to in paragraph (a)(4)(ii)(*b*) of this section, and no sample referred to in paragraph (a)(4)(iii)(*b*) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay*—(1) *Potency.* Using the phenoxymethyl penicillin working standard as the standard of comparison, proceed as directed in § 440.155c(b)(1), except prepare the sample as follows: Reconstitute the product as directed in the labeling, remove a suitable representative aliquot, and dilute with sufficient 1 percent phosphate buffer, pH 6.0, to give a concentration of 2,000 units per milliliter (estimated) if the iodometric assay method is used, or 1.0 unit per milliliter (estimated) if the bioassay method is used. Its potency is satisfactory if it contains not less than 85 percent of the number of units that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 440.74a(b)(5).

§ 440.173 Potassium phenoxymethyl penicillin oral dosage forms.

§ 440.173a Potassium phenoxymethyl penicillin capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Potassium phenoxymethyl penicillin capsules are composed of potassium phenoxymethyl penicillin and 1 or more suitable lubricants and fillers. Each capsule contains 400,000 or 800,000 units of potassium phenoxymethyl penicillin (equivalent to 250 or 500 milligrams of phenoxymethyl penicillin). The potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams or units of phenoxymethyl penicillin that it is represented to contain. Its loss on drying is not more than 2.0 percent. The potassium phenoxymethyl penicillin conforms to the standards prescribed by § 440.73(a) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The potassium phenoxymethyl penicillin used in making the batch for potency, safety, moisture, pH, crystallinity, and phenoxymethyl penicillin content.

(*b*) The batch for potency and loss on drying.

(ii) Samples required:

(a) The potassium phenoxymethyl penicillin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Place a representative number of capsules into a high-speed glass blender jar containing sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Blend 3 to 5 minutes.

(ii) *Assay procedures.* Using the phenoxymethyl penicillin working standard as the standard of comparison, assay by either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(a) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 unit of phenoxymethyl penicillin per milliliter (estimated).

(b) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

§ 440.173b Potassium phenoxymethyl penicillin chewable wafers.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Potassium phenoxymethyl penicillin chewable wafers are wafers composed of potassium phenoxymethyl penicillin with suitable diluents, binders, buffers, colorings, and flavorings. Each wafer contains either 200,000 units or 400,000 units of phenoxymethyl penicillin. The moisture content is not more than 1.5 percent. The potassium phenoxymethyl penicillin used conforms to the standards prescribed by § 440.73 (a)(1). Each other ingredient used, if its name in recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* In all cases the immediate container shall be a tight container as defined by the U.S.P. and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused that are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of wafers in such batch, the number of wafers packaged into dispensing-size containers during each day's packaging operations, the number of units in each wafer, the date on which the latest assay of the drug comprising such batch was completed, the date (unless submitted previously) on which the latest assay of the potassium phenoxymethyl penicillin used in making such batch was completed, the quantity of each ingredient used in making the batch, and a statement that such ingredient conforms to the requirements prescribed therefor, if any, by this section.

(ii) Except as otherwise provided in paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request results of the tests and assays listed after each of the following made by him on an accurately representative sample of:

(*a*) The batch:

(*1*) If the person who requests certification is the manufacturer of the batch: Average potency and average moisture of wafers collected during the time of compressing the batch; and, unless the wafers are packaged into dispensing-size containers immediately after they are compressed, average moisture of wafers collected during each day of packaging the batch.

(*2*) If the person who requests certification is not the manufacturer of the batch: Average potency and average moisture of wafers collected during each day the wafers are being packaged into dispensing-size containers.

(*b*) The potassium phenoxymethyl penicillin used in making the batch: Potency, toxicity, moisture, pH, crystallinity, and phenoxymethyl penicillin content.

(iii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch:

(*1*) If the person who requests certification is the manufacturer of the batch: One wafer for each 5,000 wafers in the batch, but in no case less than 30 wafers collected by taking single wafers at such intervals throughout the entire time of compressing the batch that the quantities compressed during the intervals are approximately equal;

(*2*) If, after compressing, such person packages the batch into dispensing-size containers: 20 wafers collected at equal intervals during each day the wafers are being packaged, except that this sample is not required if the wafers are packaged immediately after they are compressed; or

(*3*) If the person who requests certification is not the manufacturer of the batch (for the purposes of certification, a batch shall be that number of wafers filled by such person into dispensing-size containers during each day's packaging operations): One wafer for each 5,000 wafers in the batch, but in no case less than 30 wafers collected by taking single wafers at such intervals throughout each day of packaging the wafers that the quantities packaged during the intervals are approximately equal.

(*b*) The potassium phenoxymethyl penicillin used in making the batch: 10 packages, each containing equal portions of not less than 300 milligrams.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(iv) The result referred to in paragraph (a)(4)(ii)(*b*) of this section and the sample referred to in paragraph (a)(4)(iii)(*b*) of this section are not required if such result and sample have been previously submitted.

(b) *Tests and methods of assay*—(1) *Potency.* Using the phenoxymethyl penicillin working standard as the standard of comparison proceed as directed in § 440.80a(b)(1) except in lieu of the directions in § 440.80a(b)(1)(iv), prepare the sample as follows: Grind a representative number of wafers (usually 5 to 12) using a mortar and pestle. Transfer all the powder, or a weighted aliquot of the powder, to an appropriate volumetric flask, made to mark by adding sufficient 1.0-percent phosphate buffer, pH 6.0, and shake on a mechanical shaker for not less than 45 minutes. Remove from shaker and let stand in a refrigerator overnight. Filter through a Whatman No. 1 filter paper. Remove an aliquot of the filtrate, and make the proper estimated dilutions to the reference concentration in 1.0-percent phosphate buffer, pH, 6.0. The potency of potassium phenoxymethyl penicillin chewable wafers is satisfactory if they contain not less than 90 percent and not more than 125 percent of the number of units of phenoxymethyl penicillin that they are represented to contain.

(2) *Moisture.* Use four wafers and proceed as directed in § 440.80a(b)(5)(i).

§ 440.174 Procaine penicillin in oil capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Procaine penicillin in oil capsules are capsules composed of procaine penicillin, aluminum monostearate, and sesame oil or peanut oil, enclosed in a suitable and harmless soft gelatin capsule. The potency of each capsule is not less than 200,000 units. Its moisture content is not more than 2.5 percent. The procaine penicillin used conforms to the standards prescribed by § 440.74a(a)(1) except § 440.74a(a)(1)(ii) and (iii). Each other ingredient used if its name is recognized in the U.S.P. or N.F. conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* In all cases the immediate container shall be a tight container as defined by the U.S.P. and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packag-

ing, storage, and distribution practice shall be disregarded.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on the outside wrapper or container and the immediate container the statement "Expiration date _____", the blank being filled in with the date that is 18 months after the month during which the batch was certified.

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless it was previously submitted) the date on which the latest assay of the penicillin used in making such batch was completed, the number of units in each capsule, the quantity of each ingredient used in making the batch, the date on which the latest assay of the drug comprising such batch was completed, and a statement that each ingredient used in making the batch conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided in paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request results of the tests and assays listed after each of the following, made by him on an accurately representative sample of:

(*a*) The batch; average potency per capsule and average moisture.

(*b*) The penicillin used in making the batch; potency, toxicity, moisture, pH, penicillin K content (unless it is procaine penicillin G), crystallinity, and the procaine penicillin G content.

(iii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch; 1 capsule for each 5,000 capsules in the batch, but in no case less than 30 capsules, collected by taking single capsules at such intervals throughout the entire time of capsuling the batch that the quantities capsuled during the intervals are approximately equal.

(*b*) The penicillin used in making the batch; 10 packages each containing approximately equal portions of not less than 300 milligrams, packaged in accordance with the requirements of § 440.74a(a)(2).

(*c*) In case of an initial request for certification, the sesame oil or peanut oil and each other ingredient used in making the batch; 1 package of each containing, respectively, approximately 250 grams and approximately 5 grams.

(iv) No result referred to in paragraph (a)(4)(ii)(*b*) of this section, and no sample referred to in paragraph (a)(4)(iii)(*b*) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 440.80a(b)(1), except paragraph (b)(1)(ix) of that section, and in lieu of the directions in § 440.80a(b)(1)(iv) prepare the sample as follows: Place 12 capsules in a blending jar containing 40 milliliters of absolute alcohol and 200 milliliters of 1.0 percent phosphate buffer, pH 6.0. After blending for 1 minute with a high-speed blender, add 260 milliliters of 1.0 percent phosphate buffer, pH 6.0. Blend again for 1 minute and make the proper estimated dilutions in 1-percent phosphate buffer at pH 6.0. Its potency is satisfactory if it contains not less than 85 percent of the number of units per capsule that it is represented to contain.

(2) *Moisture.* Using an accurately weighed sample of approximately 1 gram of the capsule contents, proceed as directed in § 436.500(c).

§ 440.180 Penicillin oral dosage forms.

§ 440.180a Penicillin tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Penicillin tablets are tablets composed of sodium penicillin, calcium penicillin, potassium penicillin, crystalline penicillin O, crystalline phenoxymethyl penicillin, crystalline potassium phenoxymethyl penicillin, benzathine penicillin G, or procaine penicillin, with or without one or more suitable and harmless buffer substances, diluents, binders, lubricants, colorings, and flavoring. If intended for veterinary use, they may contain one or more suitable sulfonamides. The potency of each tablet is not less than 50,000 units, and if it is less than 100,000 units it is unscored. Its moisture content is not more than 1 percent if it contains sodium penicillin, calcium penicillin, potassium penicillin, or crystalline penicillin O; not more than 2 percent if it contains procaine penicillin; not more than 3 percent if it contains crystalline phenoxymethyl penicillin; and not more than 8 percent if it contains benzathine penicillin G. If it contains crystalline potassium phenoxymethyl penicillin, its moisture content is not more than 1.5 percent unless the person who requests certification has submitted to the Commissioner information adequate to prove that his drug is stable when it has a moisture content not exceeding 3 percent. Tablets not exceeding 15 millimeters, or not intended only for preparing solutions, shall disintegrate within 1 hour, unless the manufacturer has submitted blood-concentration data adequate to prove that his drug is absorbed satisfactorily from the gastrointestinal tract and such data have been accepted by the Commissioner. In such cases, the time required for the tablets to disintegrate shall not exceed that of the tablets used in such studies. The penicillin used conforms to the standards prescribed for such drug by the regulations in this chapter, except the standards for sterility and pyrogens. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* Unless each penicillin tablet is enclosed in a foil or plastic film and such enclosure is a tight container as defined by the U.S.P., except the provision that it shall be capable of tight reclosure, the immediate container shall be a tight container as so defined. The immediate container may also contain a desiccant separated from the tablets by a plug of cotton or other like material. The composition of the immediate container, or of the foil or film enclosure, shall be such as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded. If the penicillin tablets are freely soluble, each immediate container may be packaged in combination with one immediate container of a suitable and harmless aqueous vehicle with or without one or more suitable sulfonamides.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on the outside wrapper or container and the immediate container, as hereinafter indicated, the following:

(i) If the batch contains buffer substances, the name of each substance.

(ii) The statement "Expiration date _____", the blank being filed in with one of the following dates after the month during which the batch was certified:

(*a*) If a crystalline penicillin is not used, 12 months.

(*b*) [Reserved]

(*c*) If a phenoxymethyl penicillin or potassium phenoxymethyl penicillin is used, 24 months.

(*d*) If a crystalline penicillin other than a phenoxymethyl penicillin is used, 36 months.

(*e*) In lieu of the expiration date prescribed in paragraph (a)(3)(ii)(*a*) (*c*) and (*d*) of this section, if the person requesting certification has submitted to the Commissioner results of tests and assays that show such drug as prepared by him is stable for 24, 36, 48, or 60 months, such date may be used for such drug.

(iii) If it contains, in addition to penicillin, one or more of the other active ingredients specified in paragraph (a)(1) of this section, after the name "penicillin tablets", wherever it appears, the words "with _____ (the blank being filled in with the established name of each other ingredient)", in juxtaposition with such name.

(4) *Requests for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of tablets in such batch, the number of tablets of the batch packaged into dispensing-size containers during each day's packaging operations, the number of units in each tablet, the date on which the latest assay of the drug comprising such batch was completed, the date (unless submitted previously) on which the latest assay of the penicillin used in making such batch was completed, the quantity of each ingredient

used in making the batch, and a statement that such ingredient conforms to the requirements prescribed therefor, if any, by this section.

(ii) Except as otherwise provided in paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request results of the tests and assays listed after each of the following, made by him on an accurately representative sample of:

(*a*) The batch:

(*1*) If the person who requests certification is the manufacturer of the batch: Average potency, average moisture, and, if required by paragraph (a)(1) of this section, disintegration time of tablets collected during the time of tableting the batch; and, unless the tablets are packaged into dispensing-size containers immediately after they are compressed or the manufacturer has submitted to the Commissioner, and it has been accepted, information adequate to prove that such tests are not necessary, average moisture of tablets collected during each day of packaging the batch.

(*2*) If the person who requests certification is not the manufacturer of the batch: Average potency, average moisture, and, if required by paragraph (a)(1) of this section, disintegration time of tablets collected during each day the tablets are being packaged into dispensing-size containers.

(*b*) The penicillin used in making the batch; potency, toxicity, moisture, pH, penicillin K content (unless it is crystalline penicillin G, crystalline penicillin O, crystalline phenoxymethyl penicillin, or crystalline potassium phenoxymethyl penicillin), crystallinity if it is crystalline penicillin; heat stability if it is crystalline sodium penicillin, potassium penicillin, or crystalline penicillin O; the penicillin G content if it is a penicillin G or crystalline penicillin O; the phenoxymethyl penicillin content if it is a phenoxymethyl penicillin; and the penicillin O content if it is crystalline penicillin O.

(iii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch:

(*1*) For potency and moisture:

(*i*) If the person who requests certification is the manufacturer of the batch: One tablet for each 5,000 tablets in the batch, but in no case less than 30 tablets collected by taking single tablets at such intervals throughout the entire time of tableting the batch that the quantities tableted during the intervals are approximately equal;

(*ii*) If, after tableting, such person packaged the batch into dispensing-size containers: 20 tablets collected at equal intervals during each day the tablets are being packaged, except that this sample is not required if the tablets are packaged immediately after they are compressed or if the manufacturer has been exempted by the Commissioner from such requirement; or

(*iii*) If the person who requests certification is not the manufacturer of the batch (for the purposes of certification, a batch shall be that number of tablets filled by such person into dispensing-size containers during each day's packaging operations): One tablet for each 5,000 tablets in the batch, but in no case less than 30 tablets collected by taking single tablets at such intervals throughout each day of packaging the tablets that the quantities packaged during the intervals are approximately equal.

(*2*) For disintegration time: 6 tablets.

(*b*) The penicillin used in making the batch; 6 packages, or in the case of crystalline penicillin 10 packages, each containing approximately equal portions of not less than 60 milligrams, except that if it is procaine penicillin, benzathine penicillin G, phenoxymethyl penicillin, or potassium phenoxymethyl penicillin each package shall contain not less than 300 milligrams, packaged in accordance with the requirements of § 440.80a(a)(2), 440.74a(a)(2), 440.55a(a)(2), or 440.71(a)(2).

(*c*) In case of an initial request, each other substance used in making the batch; one package of each, containing approximately 5 grams, and if the batch or any part thereof is to be packaged in combination with an aqueous vehicle, or when any change is made in the composition of such vehicle, five packages of the vehicle included in the combination.

(iv) No result referred to in paragraph (a)(4)(ii)(*b*) of this section, and no sample referred to in paragraph (a)(4)(iii)(*b*) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Tablets that do not contain procaine penicillin G, benzathine penicillin G, Phenoxymethyl penicillin, or potassium phenoxymethyl penicillin.* Proceed as directed in § 440.80a(b)(1), except § 440.80a(b)(1)(ix), and in lieu of the directions in § 440.80a(b)(1)(iv) prepare the sample by one of the following methods:

(*a*) *Grinding.* Grind a representative number of tablets (usually 5 to 12), using a mortar and pestle. Transfer all the powder or a weighed aliquot of the powder to an appropriate volumetric flask and make to mark by adding sufficient phosphate buffer, and shake thoroughly. Make the proper estimated dilutions in 1 percent phosphate buffer, pH 6.0.

(*b*) *Blending.* Place a representative number of tablets (usually 5 to 12) in a blending jar and add thereto approximately 200 milliliters of a 500-milliliter quantity of 1 percent phosphate buffer, pH 6.0. After blending for 1 minute with a high-speed blender, add the remainder of the 500 milliliters of buffer. Blend again for 1 minute and make the proper estimated dilutions in 1 percent phosphate buffer, pH 6.0.

(*c*) *Dissolving.* If the tablets dissolve readily, dissolve a representative number of tablets (usually 5 to 12) in 1 percent phosphate buffer, pH 6.0, and make the proper estimated dilutions in 1 percent phosphate buffer, pH 6.0.

(ii) *Tablets that contain benzathine penicillin G.* Proceed as directed in § 440.80a(b)(1), except § 440.80a(b)(1)(ix), and in lieu of the direction in § 440.80a(b)(1)(iv) prepare the sample as follows: Grind 6 tablets, using a mortar and pestle, and add sufficient formamide or dimethylformamide (previously adjusted, if necessary, to pH 6 to 7 with a few drops of concentrated H_2SO_4 per liter) to give a concentration of not more than 15,000 units per milliliter. Allow to stand for ½ hour, with frequent agitation, then make the proper estimated dilutions in 1 percent phosphate buffer at pH 6.0. The sample may also be prepared by one of the following methods: Place 6 tablets in a blending jar containing 100 milliliters of a 500-milliliter quantity of redistilled methyl alcohol. After blending for 1 minute with a high-speed blender, add the remainder of the 500-milliliter quantity of redistilled methyl alcohol. Blend again and make the proper estimated dilutions, using 1.0 percent phosphate buffer, pH 6.0, or grind a representative number of tablets (usually 5 to 12), using a mortar and pestle. Add sufficient redistilled methyl alcohol to the powder, or a weighed aliquot of the powder, to give a concentration of 2,000 units to 5,000 units per milliliter. Allow to stand for ½ hour, with frequent agitation, then dilute with sufficient 1.0 percent phosphate buffer, pH 6.0, to give a stock solution of not less than 200 units per milliliter. Make the proper estimated dilutions, using 1.0 percent phosphate buffer, pH 6.0, to give a concentration of 1.0 unit per milliliter. If the iodometric assay method is used, proceed as directed in § 440.155b(b)(1)(i).

(iii) *Tablets that contain procaine penicillin G.* Proceed as directed in § 440.80a(b)(1) except § 440.80a(b)(1)(ix), and in lieu of the directions in § 440.80a(b)(1)(iv) prepare the sample as follows: Grind a representative number of tablets (usually 5 to 12) using a mortar and pestle. To the powder, or a weighed aliquot of the powder, add sufficient redistilled methanol to give a concentration of 10,000 to 100,000 units per milliliter. Agitate thoroughly and dilute further with sufficient 1 percent phosphate buffer, pH 6.0, to give a concentration of 2,000 units per milliliter (estimated) if the iodometric assay method is used, or a concentration of 1.0 unit per milliliter (estimated) if the bioassay method is used.

(iv) *Tablets that contain phenoxymethyl penicillin.* Using the phenoxymethyl penicillin working standard as the standard of comparison, proceed as directed in paragraph (b)(1)(iii) of this section.

(v) *Tablets that contain potassium phenoxymethyl penicillin.* Using the phenoxymethyl penicillin working standard as the standard of comparison, proceed as directed in paragraph (b)(1)(i) of this section.

The average potency of penicillin tablets is satisfactory if they contain not less than 85 percent of the number of units per tablet that they are represented to contain.

(2) *Moisture.* Use 4 tablets and proceed as directed in § 440.80a(b)(5)(i), except that if they contain benzathine

penicillin G or phenoxymethyl penicillin proceed as directed in § 440.74a(b)(5).

(3) *Disintegration time*—(i) *Uncoated tablets.* Proceed as directed in the U.S.P., using simulated gastric fluid test solution. The tablets are satisfactory if they disintegrate within 1 hour.

(ii) *Plain coated tablets.* Proceed as directed in the U.S.P. If the tablets have not disintegrated after 30 minutes in the gastric fluid, use simulated intestinal fluid for an additional 30 minutes. The tablets are satisfactory if they disintegrate within 1 hour.

§ 440.180b Penicillin-streptomycin tablets; penicillin-dihydrostreptomycin tablets.

(a) *Requirements for certification*—(1) Penicillin-streptomycin tablets and penicillin - dihydrostreptomycin tablets conform to all requirements and are subject to all procedures prescribed by § 440.180a(a) for penicillin tablets, except that:

(i) Each tablet contains not less than 20 milligrams of streptomycin or dihydrostreptomycin. The streptomycin or dihydrostreptomycin used conforms to the standards prescribed by § 444.70a(a)(1) or § 444.10a(a) of this chapter, except the standards for sterility, pyrogens, and histamine content.

(ii) In lieu of the labeling prescribed for penicillin tablets by § 440.180a(a)(3)(i)(*b*), each package shall bear on the outside wrapper or container and the immediate container the statement "Expiration date _____", the blank being filled in with the date that is 18 months after the month during which the batch was certified.

(iii) In addition to complying with the requirements of § 440.180a(a)(4), a person who requests certification of a batch of penicillin-streptomycin tablets or penicillin-dihydrostreptomycin tablets shall submit with his request a statement showing the batch mark and (unless it was previously submitted) the results and the date of the latest tests and assays of the streptomycin or dihydrostreptomycin used in making the batch for potency, toxicity, moisture, pH, streptomycin content if it is dihydrostreptomycin, and crystallinity if it is crystalline dihydrostreptomycin and the number of milligrams of streptomycin or dihydrostreptomycin in each tablet of the batch. He shall also submit in connection with his request (unless it was previously submitted) a sample consisting of five packages containing approximately equal portions of not less than 0.5 gram each of the streptomycin or dihydrostreptomycin used in making the batch, packaged in accordance with the requirements of § 444.70a(a)(2) of this chapter.

(2) [Reserved.]

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Penicillin content.* Proceed as directed in § 440.180a(b)(1). Its content of pencillin is satisfactory if it contains not less than 85 percent of the number of units that it is represented to contain.

(ii) *Streptomycin content.* Proceed as directed in § 141a.36(a)(2). Its content of streptomycin is satisfactory if it contains not less than 85 percent of the number of milligrams it is represented to contain.

(iii) *Dihydrostreptomycin content.* Proceed as directed in § 141a.36(a)(3) of this chapter. Its content of dihydrostreptomycin is satisfactory if it contains not less than 85 percent of the number of milligrams it is represented to contain.

(2) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i).

(3) *Disintegration time.* Proceed as directed in § 440.180a(b)(3).

§ 440.180c Crystalline penicillin G capsules (crystalline penicillin G potassium capsules, crystalline penicillin G sodium capsules).

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Crystalline capsules are crystalline penicillin G, penicillin C, sodium or potassium, with or without one or more suitable and harmless buffer substances, and vegetable oils, enclosed in suitable and harmless gelatin capsules. The potency of each capsule is not less than 50,000 units. The moisture content is not more than 1.5 percent unless the person who requests certification has submitted information adequate to prove that his drug is stable for that period of time covered by its expiration date when it has a moisture content of more than 1.5 percent; but in no case shall its moisture content be more than 4.0 percent. The crystalline penicillin G used conforms to the requirements of § 440.80a(a)(1), except § 440.80a(a)(1)(ii) and (iv). Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging; labeling; request for certification, samples.* Crystalline penicillin G capsules conform to all requirements and procedures prescribed for crystalline penicillin G tablets by § 440.180a(a)(2), (3), and (4), except that:

(i) If it is for use in the preparation of oral solutions, each package shall bear on its outside wrapper or container and the immediate container a statement that contains adequate directions for preparing such solutions and a statement that such solutions should be used immediately.

(ii) [Reserved]

(iii) It shall be labeled with an expiration date that is 36 months after the month during which the batch was certified, except that the date may be 48 or 60 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time such drug as prepared by him complies with the standards prescribed by paragraph (a)(1) of this section.

(b) *Tests and methods of assay*—(1) *Potency.* Use the contents of a representative number of capsules (usually 5 to 12) and proceed as directed in § 440.80a(b)(1), except § 440.80a(b)(1)(ix), and except if it contains a vegetable oil, prepare the sample as follows: Place the capsules in a blending jar containing 1.0 milliliter of polysorbate 80 and sufficient 1 percent phosphate buffer, pH 6.0, to give a volume of 250 milliliters. Using a high-speed blender, blend for 3 to 5 minutes, then add 250 milliliters of buffer and blend for an additional minute. Make further dilutions to 1 unit per milliliter (estimated) with 1 percent phosphate buffer, pH 6.0. The average potency of capsules crystalline penicillin G is satisfactory if it is not less than 85 percent of the number of units per capsule they are represented to contain.

(2) *Moisture.* Use the contents of 4 capsules and proceed as directed in § 440.80a(b)(5)(i), except if it contains a vegetable oil proceed as directed in § 436.500(c) of this chapter.

§ 440.180d Penicillin and novobiocin capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Capsules penicillin and novobiocin are crystalline penicillin G sodium or potassium and crystalline monosodium novobiocin, with or without one or more suitable and harmless buffer substances, lubricants, colorings, and flavorings, enclosed in a suitable and harmless gelatin capsule. Each capsule contains not less than 125,000 units of penicillin and not less than 125 milligrams of novobiocin. The moisture content is not more than 4.0 percent. The crystalline penicillin G used conforms to the requirements of § 440.80a(a)(1), except § 440.80a(a)(1)(ii) and (iv). The crystalline monosodium novobiocin used is produced by the growth of *Streptomyces niveus* has a potency of not less than 750 micrograms per milligram, is nontoxic, has a moisture content of not more than 6.0 percent, and the pH in a saturated aqueous solution is not less than 6.5 and not more than 8.5. Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging; labeling; request for certification, samples.* Capsules penicillin and novobiocin conform to all requirements and procedures prescribed for crystalline penicillin G tablets by § 440.180a(a)(2), (3) and (4) except with respect to disintegration time, and except that:

(i) The expiration date shall be the date that is 18 months after the month during which the batch was certified, except that if the person who requests certification has submitted to the Commissioner results of tests and assays that show such drug as prepared by him is stable for 24 months, 36 months, or 48 months, such date may be used for such drug.

(ii) In addition to complying with the requirements of § 440.180a(a)(4), a person who requests certification of a batch shall submit with his request a statement showing the batch mark and (unless they were previously submitted) the results and the date of the latest tests and assays of the novobiocin used in making the batch for potency, toxicity, pH, moisture, and crystallinity. He shall

also submit in connection with his request (unless previously submitted) a sample consisting of 10 packages, each containing approximately equal portions of not less than 300 milligrams of the novobiocin used in making the batch.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Penicillin content*—(*a*) *Plate assay* Proceed as directed in § 440.80a(b)(1), with the following exceptions:

(*1*) In lieu of the directions prescribed by § 440.80a(b)(1)(iv), prepare the sample by dissolving the contents of a representative number of capsules (usually 5 to 12) in sufficient 1.0-percent potassium phosphate buffer (pH 6.0) to give a stock solution of convenient concentration.

(*2*) In lieu of the directions prescribed by § 440.80a(b)(1)(v), use as the test organism the novobiocin-resistant strain of *Staphylococcus aureus* (ATCC 12692).[1] Maintain the organism on agar containing 10 micrograms of novobiocin per milliliter. Prepare an organism suspension by washing the organism from an agar slant (which has been incubated for 24 hours at 32° C.–35° C.) with 3.0 milliliters of sterile physiological saline solution onto a large surface of agar containing 10 micrograms of novobiocin per milliliter. A Roux bottle containing 300 milliliters of agar is satisfactory. Incubate 24 hours at 32° C.–35° C. Wash the resulting growth from the agar surface with sufficient sterile physiological saline solution, so that a tenfold dilution of the bulk suspension will give 20-percent light transmission, using a suitable photoelectric colorimeter with a 650-millimicron filter and an 18-millimeter-diameter test tube as an absorption cell. Run test plates to determine the quantity of bulk suspension (usually 1 milliliter) that should be added to each 100 milliliters of agar to give clear, sharp zones of inhibition of appropriate size.

(*b*) *Iodometric assay.* Proceed as directed in § 440.80a(b)(5)(iv)(*a*), with the following exceptions: Use the contents of a representative number of capsules (usually 5 to 12) dissolved in sufficient 1.0-percent potassium phosphate buffer (pH 6.0) to give a concentration of 2,000 units per milliliter (estimated). In the blank determination add 1 drop of 1.2 *N* HCl to the 2.0-milliliter aliquot of the assay solution immediately before the addition of the 10.0 milliliters of 0.01*N* iodine. Prepare the inactivated solution as follows: To 5.0 milliliters of the assay solution add 10.0 milliliters of 1*N* NaOH and allow to stand at room temperature. After 15 minutes add 10.0 milliliters of 1.2*N* HCl, mix well, and filter through filter paper. To 10.0 milliliters of clear filtrate add 10.0 milliliters of 0.01*N* iodine.

Its content of penicillin is satisfactory if it contains not less than 85 percent of the number of units per capsule that it is represented to contain.

(ii) *Novobiocin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar containing sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. To an aliquot add sufficient penicillinase to inactivate the penicillin, further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6) to give a reference concentration of 0.5 microgram of novobiocin per milliliter (estimated) and allow to stand for one-half hour at 37° C. Its content of novobiocin is satisfactory if it contains not less than 85 percent of the number of milligrams per capsule that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i).

(3) *Novobiocin used in making the capsules*—(i) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 0.5 microgram of novobiocin per milliliter (estimated.)

(*a*) *Cylinders* (*cups*). Use cylinders described in § 440.80a(b)(1)(i).

(*b*) *Culture media.* (*1*) Use the nutrient agar described in § 440.80a(b)(1)(ii)(*a*) for the seed layer and for carrying the organism.

(*2*) Use the nutrient agar described in § 440.80a(b)(1)(ii)(*b*) for the base layer.

(*c*) *Working standard.* Dissolve a suitable weighed quantity (30 to 50 milligrams) of the working standard (obtained from the Food and Drug Administration) dried as described in § 440.80a(b)(5)(i) in 2 milliliters of ethyl alcohol and then add sufficient 0.1*M* potassium phosphate buffer (pH 8.0) to give a concentration of 1,000 micrograms per milliliter. This stock solution may be kept for 30 days when stored under refrigeration.

(*d*) *Preparation of sample.* Dissolve in sufficient 0.1*M* potassium phosphate buffer (pH 8.0) to give a convenient stock solution. Further dilute in 1.0-percent potassium phosphate buffer (pH 6.0) to give a final concentration of 0.5 microgram per milliliter (estimated).

(*e*) *Prepartion of test organism.* The test organism is *Staphylococcus epidermidis* (American Type Culture Collection 12228)[1] which is maintained on slants of agar described under paragraph (b)(3)(i)(*b*)(*1*) of this section. Wash the organism from the agar slant with 3 milliliters of sterile physiological saline solution onto a large agar surface such as that provided by a Roux bottle containing 300 milliliters of the agar described in paragraph (b)(3)(i)(*b*)(*1*) of this section. Spread the suspension of organisms over the entire agar surface with the aid of sterile glass beads. Incubate for 24 hours at 32° C.–35° C., and then wash the resulting growth from the agar surface with sufficient sterile physiological saline solution so that a tenfold dilution of this bulk suspension will give 15-percent light transmission, using a suitable photoelectric colorimeter with a 650-millimicron filter and an 18-millimeter-diameter test tube as an absorption cell. Run test plates to determine the quantity of the bulk suspension (usually 1.5 milliliters) that should be added to each 100 milliliters of agar to give clear, sharp zones of inhibition of appropriate size.

(*f*) *Preparation of plates.* Add 21 milliliters of the agar prepared as described in paragraph (b)(3)(i)(*b*)(*2*) of this section to each Petri dish (20 millimetrs x 100 millimeters). Distribute the agar evenly in the plates and allow it to harden. Use the plates the same day they are prepared. Melt a sufficient amount of the agar described in paragraph (b)(3)(i)(*b*)(*1*) of this section, cool to 48° C., add the proper amount of the test organism as described above, and mix thoroughly. Add 4 milliliters of this inoculated agar to each Petri dish. Distribute the agar evenly in the plates, cover with porcelain covers glazed on the outside, and allow to harden. After the agar has hardened, place 6 cylinders on the agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius.

(*g*) *Standard curve.* Prepare the daily standard curve by further diluting the 1,000 μg. per milliliter stock solution in 1 percent potassium phosphate buffer (pH 6.0), to obtain concentrations of 0.32, 0.4, 0.5, 0.63, and 078 μg. per milliliter. Use three plates for the determination of each point on the curve, except the 0.5 μg. per milliliter concentration, a total of 12 plates. On each of three plates fill three cylinders with the 0.5 μg. per milliliter standard, and fill the other three cylinders with the concentration under test. Thus, there will be thirty-six ½-μg. determinations and nine determinations for each of the other points on the curve. After the plates have incubated, read the diameters of the circles of inhibition. Average the readings of the 0.5 μg. per milliliter concentration and the readings of the point tested for each set of three plates, and average also all 36 readings of the 0.5 μg. per milliliter concentrations. The average of the 36 readings of the 0.5 μg. per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 0.5 μg. per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 0.4 μg. concentration, the average of the 36 readings of the 0.5 μg. concentration is 20.0 millimeters, and in correcting the 0.4 μg. concentration, of this set of three plates is 19.8 millimeters, the correction is +0.2 millimeter. If the average reading of the 0.4 μg. concentration of these same three plates is 19.0 millimeters, the corrected value is 19.2 millimeters. Plot these corrected values, including the average of the 0.5 μg. per milliliter concentration, on semilog paper, placing the concentration in

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

micrograms per milliliter on the logarithmic scale and the diameter of the zone of inhibition on the arithmetic scale. Draw the standard curve through these points either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

L = corrected zone diameter for the lowest concentration of the standard curve.
H = corrected zone diameter for the highest concentration of the standard curve.
c = average zone diameter of 36 readings of the 0.5 μg. per milliliter standard.
a, b, d, e = corrected average values for the 0.32, 0.4, 0.63, and 0.78 μg. per milliliter standard solutions, respectively.

Plot the values obtained for L and H and connect with a straightline.

(*h*) *Assay.* Use 3 plates for each sample. Fill 3 cylinders on each plate with the standard 0.5 microgram per milliliter solution and 3 cylinders with the 0.5 microgram per milliliter (estimated) sample, alternating standard and sample. Incubate all plates, including those containing the standard curve, at 32° C.–35° C. overnight, and measure the diameter of each circle of inhibition. To estimate the potency of the sample, average the zone readings of the standard and the zone readings of the sample on the 3 plates used. If the sample gives a larger zone size than the average of the standard, add the difference between them to the 0.5 microgram per milliliter zone on the standard curve. If the average sample value is lower than the standard value, subtract the difference between them from the 0.5 microgram per milliliter value on the standard curve. From the curve, read the potencies corresponding to these corrected values of zone sizes.

(ii) *Toxicity.* Proceed as directed in § 440.80a(b)(4), except use physiological salt solution as the diluent, and inject 0.5 milliliter of a solution containing 4 milligrams per milliliter.

(iii) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i).

(iv) *pH.* Proceed as directed in § 440.80a(b)(5)(ii), using a saturated aqueous solution (approximately 25 milligrams per milliliter) of the drug.

(v) *Crystallinity* Proceed as directed in § 440.80a(b)(5)(iii).

§ 440.180e Crystalline penicillin G oral suspension, crystalline penicillin G sodium oral suspension, potassium penicillin G oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Crystalline penicillin G oral suspension is crystalline penicillin G sodium or potassium penicillin G and one or more suitable and harmless suspending or dispersing agents and preservatives, with or without suitable and harmless buffer substances, colorings, and flavorings, suspended in a suitable and harmless vehicle. Its potency is not less than 20,000 units per milliliter. Its moisture content is not more than 1.0 percent. The crystalline penicillin used conforms to § 440.80a(a)(1), except § 440.80a(a)(1)(ii) and (iv). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* The immediate container shall be a tight container as defined by the U.S.P., and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused that are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its outside wrapper or container and the immediate container the following:

(i) If the batch contains buffer substances, the name of each such substance.

(ii) The statement "Expiration date _____", the blank being filled in with the date that is 24 months after the month during which the batch was certified, except that if the person who requests certification has submitted to the Commissioner results of tests and assays that show such drug as prepared by him is stable for 36 months, such date may be used for such drug.

(iii) If it contains, in addition to penicillin, one or more of the other active ingredients specified in paragraph (a)(1) of this section, after the name "crystalline penicillin G oral suspension", wherever it appears, the words "with _____ (the blank being filled in with the established name of each such other ingredient)", in juxtaposition with such name.

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless it was previously submitted) the date on which the latest assay of the penicillin G used in making such batch was completed, the potency per milliliter of the batch, the date on which the latest assay of the drug comprising such batch was completed, the quantity of each ingredient used in making the batch, and a statement that each such ingredient conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided in paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request results of the tests and assays listed after each of the following, made by him on an accurately representative sample of:

(*a*) The batch; average potency per milliliter and moisture.

(*b*) The penicillin G used in making the batch; potency, toxicity, pH, moisture, crystallinity, and the penicillin G content.

(iii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch; 1 immediate container for each 5,000 immediate containers in the batch, but in no case less than 5 immediate containers, collected by taking single immediate containers at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(*b*) The penicillin G used in making the batch; 10 packages, each containing approximately equal portions of not less than 60 milligrams, packaged in accordance with the requirements of § 440.80a(a)(2).

(*c*) In case of an initial request for certification, each other ingredient used in making the batch; 1 package of each containing approximately 5.0 grams.

(iv) No result referred to in paragraph (a)(4)(ii)(*b*) of this section, and no sample referred to in paragraph (a)(4)(iii)(*b*) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 440.80a(b)(1), except § 440.80a(b)(1)(ix), and in lieu of the directions in § 440.80a(b)(1)(iv), prepare the sample as follows: By means of a hypodermic syringe, introduce 5 milliliters of the well-shaken test sample into a high-speed blender containing 244 milliliters of 1-percent phosphate buffer at pH 6.0 and 1.0 milliliter of polysorbate 80. Blend the mixture for 3 to 5 minutes. Make the proper estimated dilutions in 1-percent phosphate buffer at pH 6.0. Its potency is satisfactory if it contains not less than 85 percent of the number of units per milliliter that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 436.500(c).

§ 440.180f Buffered penicillin powder, penicillin powder with buffered aqueous diluent.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Buffered penicillin powder is a mixture of crystalline penicillin or procaine penicillin and suitable buffer substances, with or without the addition of one or more suitable and harmless diluents, colorings, and flavorings. Penicillin powder with buffered aqueous diluent is a packaged combination of one immediate container of crystalline penicillin or procaine penicillin, with or without suitable and harmless diluents, and one immediate container of an aqueous diluent containing suitable buffer substances and suitable and harmless preservatives, colorings, and flavorings. Its moisture content is not more than 1 percent, except if it contains procaine penicillin its moisture content is not more than 2 percent.

The crystalline penicillin used conforms to the requirements of § 440.80a(a)(1) for crystalline penicillin, except paragraph (a)(1)(ii) and (iv) of that section. The procaine penicillin used conforms to the requirements of § 440.74a (a)(1), except paragraph (a)(1)(ii) and (iii) of that section. Each other substance used, if its name is recognized in the U.S.P. and N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* In all cases the immediate container of buffered penicillin power shall be a tight container as defined by the U.S.P. The composition of the immediate container shall be such as will not cause any change in the strength, quality, or purity of the contents beyond any limits therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded. Each immediate container may be packaged in combination with a container of a suitable and harmless aqueous vehicle.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its label and labeling, as hereinafter indicated. the following:

(i) On the otuside wrapper or container and the immediate container:

(*a*) A statement giving the method of dissolving the penicillin, if it is a packaged combination of one immediate container of buffered penicillin powder or penicillin powder and one immediate container of a vehicle.

(*b*) The statement "Expiration date ----------", the blank being filled in with the date that is 24 months after the month during which the batch was certified, except that the blank may be filled in with the date that is 36 months after the month during which the batch was certified if it is crystalline penicillin with no other ingredients; or the blank may be filled in with the date that is 48 months or 60 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time such drug as prepared by him complies with the standards prescribed by paragraph (a)(1) of this section.

(*c*) The name of each buffer substance used in making the batch.

(ii) On the label and labeling if it contains, in addition to penicillin, one or more of the other active ingredients specified in paragraph (a)(1) of this section, after the name "Buffered penicillin powder", wherever it appears, the words "with ---------- (the blank being filled in with the established name of each such other ingredient)", in juxtaposition with such name.

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch of buffered penicillin powder shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless it was previously submitted) the date on which the latest assay of the penicillin used in making such batch was completed, the number of units in each immediate container, the quantity of each ingredient used in making the batch, the date on which the latest assay of the drug comprising such batch was completed, and a statement that each ingredient used in making the batch conforms to the requirements prescribed therefor, if any, by this section.

(ii) Except as otherwise provided in paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request results of the tests and assays, listed after each of the following, made by him on an accurately representative sample of:

(*a*) The batch; if buffered penicillin powder or penicillin powder with diluents: potency and moisture; if penicillin powder with no other ingredients: potency, toxicity, moisture, pH, penicillin K content (unless it is crystalline penicillin G), crystallinity, heat stability if it is crystalline sodium or potassium penicillin, the penicillin G content if it is crystalline sodium or potassium penicillin G, and the procaine penicillin G content if it is procaine penicillin G.

(*b*) If buffered penicillin powder or penicillin powder with diluents, the penicillin used in making the batch; potency, toxicity, moisture, pH, penicillin K content (unless it is crystalline penicillin G), crystallinity, heat stability if it is crystalline sodium or potassium penicillin, the penicillin G content if it is crystalline sodium or potassium penicillin G, and the procaine penicillin G content if it is procaine penicillin G.

(iii) Except as otherwise provided by paragraph (a)(4)(iv) of this section such person shall submit in connection with this request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch:

(*1*) If it contains only penicillin and the penicillin used has been previously submitted, or it contains penicillin and other ingredients; one immediate container for each 5,000 immediate containers in the batch, but in no case less than 5 immediate containers.

(*2*) If it contains only penicillin and the penicillin has not been previously submitted; one immediate container for each 5,000 immediate containers in the batch, but in no case less than 10 immediate containers or more than 17 immediate containers.

Such sample shall be collected by taking single immediate containers at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(*b*) If buffered penicillin powder or penicillin powder with diluents, the penicillin used in making the batch; 10 packages, each containing approximately equal portions of not less than 60 milligrams if it is not procaine penicillin, and not less than 500 milligrams if it is procaine penicillin, packaged in accordance with the requirements of § 440.80a(a)(2) or 440.74a(a)(2).

(*c*) In case of an initial request for certification, each other ingredient used in making the batch; one package of each containing approximately 5 grams.

(*d*) In case of an initial request for the certification of a batch of buffered penicillin powder which is to be packaged in combination with an aqueous vehicle, or when any change is made in the composition of such aqueous vehicle; 5 packages of the vehicle included in the combination.

(iv) No result referred to in paragraph (a)(4)(ii)(*b*) of this section and no sample referred to in paragraph (a)(4)(iii)(*b*) of this section is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay*—(1) *Potency.* Reconstitute the drug as directed in its labeling and proceed as directed in § 440.80a(b)(1), except if it is procaine penicillin proceed as directed in § 440.155c(b)(1). Its potency is satisfactory if it contains not less than 90 percent of the number of units that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 440.74a(b)(5).

§ 440.182 Phenethicillin potassium oral dosage forms.

§ 440.182a Phenethicillin potassium tablets.

(a) *Requirements for certification.*—(1) *Standards of identity, strength, quality, and purity.*—Phenethicillin potassium tablets are composed of phenethicillin potassium, with or without one or more suitable and harmless buffer substances, diluents, binders, lubricants, colorings, and flavorings. Each tablet contains phenethicillin potassium equivalent to 400,000 urits of phenethicillin (equivalent to 250 milligrams of phenethicillin). Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of units or milligrams of phenethicillin that it is represented to contain. Its moisture content is not more than 2 percent. It shall disintegrate within 1 hour. The phenethicillin potassium used conforms to the standards prescribed by § 440.82(a)(1).

(2) *Labeling.*—It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The phenethicillin potassium used in making the batch for potency, safety, loss on drying, pH, phenethicillin content, L-phenethicillin potassium content, crystallinity, and identity.

(*b*) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(*a*) The phenethicillin potassium used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.204

of this chapter, preparing the sample as follows: Place a representative number of tablets into a high-speed glass blender jar containing sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. If necessary, further dilute an aliquot of the stock solution with solution 1 to obtain an assay solution containing 2,000 units per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter.

§ 440.182b Phenethicillin potassium for oral solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Phenethicillin potassium for oral solution is composed of phenethicillin potassium, with or without one or more suitable and harmless colorings, flavorings, buffer substances, and preservatives. Each milliliter contains phenethicillin potassium equivalent to 40,000 units of phenethicillin (equivalent to 25 milligrams of phenethicillin). Its potency is satisfactory if it contains not less than 90 percent and not more than 125 percent of the number of units or milligrams of phenethicillin that it is represented to contain. Its moisture content is not more than 1 percent. The phenethicillin potassium used conforms to the standards prescribed by § 440.82(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The phenethicillin potassium used in making the batch for potency, safety, loss on drying, pH, phenethicillin content, L-phenethicillin potassium content, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The phenethicillin potassium used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.204 of this chapter, preparing the sample as follows: Reconstitute as directed in the labeling. Dilute an accurately measured representative aliquot with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the prescribed concentration.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

Subpart C—Injectable Dosage Forms

§ 440.207 Sterile ampicillin trihydrate for suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile ampicillin trihydrate for suspension is a dry mixture of ampicillin trihydrate and one or more suitable and harmless buffer substances, stabilizers, suspending agents, and preservatives. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of ampicillin that it is represented to contain. It is sterile. It is nonpyrogenic. It passes the safety test. Its loss on drying is not less than 11.4 percent and not more than 14.0 percent. When reconstituted as directed in the labeling, its pH is not less than 5.0 and not more than 7.0. The ampicillin trihydrate used conforms to the standards prescribed by § 440.7a(a)(1) of this chapter.

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5 of this chapter, this drug shall be labeled "sterile ampicillin for suspension."

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The ampicillin trihydrate used in making the batch for potency, loss on drying, pH, ampicillin content, concordance, crystallinity, and identity.

(*b*) The batch for potency, sterility, pyrogens, safety, loss on drying, and pH.

(ii) Samples required:

(*a*) The ampicillin trihydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 12 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Reconsitute as directed in the labeling. Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container, or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the resultant solution with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), for the microbiological agar diffusion assay, or distilled water for the iodometric assay, to give a stock solution of convenient concentration.

(ii) *Assay procedures.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter.

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, diluting an aliquot of the stock solution with distilled water to the prescribed concentration.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except in lieu of (e)(1)(i)(*a*), prepare the sample for test as follows: From each of 10 immediate containers, aseptically transfer approximately 300 milligrams of sample into a sterile 500-milliliter Erlenmeyer flask containing approximately 400 milliliters of diluting fluid D. Add at least 200,000 Levy units[*] of penicillinase. Repeat the process using 10 additional containers. Swirl both of the stoppered flasks to completely solubilize the suspension prior to filtration and proceed as directed in paragraph (e)(1)(ii) of that section. If the formulation cannot be filtered, proceed as directed in § 436.20(e)(2) of this chapter, except use medium B in lieu of medium A.

(3) *Pyrogens.* Proceed as directed in § 436.32(f) of this chapter, using a solution containing 20 milligrams of ampicillin per milliliter.

(4) *Safety.* Proceed as directed in § 436.32 of this chapter.

(5) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using the solution obtained when the product is reconstituted as directed in the labeling.

Effective date: § 440.207 became effective on April 15, 1974, except that the nonproprietary name designated in paragraph (a)(2) shall not be effective until new labels are printed in the normal course of business or until September 15, 1974, whichever comes first. See 39 FR 13877, April 18, 1974.

§ 440.209 Sterile sodium ampicillin.

The requirements for certification and the tests and methods of assay for sterile sodium ampicillin packaged for dispensing are described in § 440.9a.

§ 440.213 Sterile disodium carbenicillin.

The requirements for certification and the tests and methods of assay for sterile disodium carbenicillin packaged for dispensing are described in § 440.13a.

§ 440.219 Sodium dicloxacillin monohydrate injectable dosage forms.

§ 440.219a Sterile sodium dicloxacillin monohydrate.

The requirements for certification and the tests and methods of assay for sterile sodium dicloxacillin monohydrate packaged for dispensing are described in § 440.19a.

§ 440.219b Sodium dicloxacillin monohydrate for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium dicloxacillin monohydrate for injection is a dry mixture of sodium dicloxacillin monohydrate and lidocaine hydrochloride packaged for dispensing. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of dicloxacillin that it is represented to contain. It is sterile. It is nonpyrogenic. It passes the safety test. Its moisture content is not more than 5 percent. When reconstituted as directed in the labeling, its pH is not

[*] One Levy unit of penicillinase inactivates 99.3 units of penicillin G in 1 hour at 25° C. and at a pH of 7.0 in a phosphate buffered solution of a pure alkali salt of penicillin G when the substrate is in sufficient concentration to maintain a zero order reaction.

less than 4.5 and not more than 7.5. The sodium dicloxacillin monohydrate used conforms to the standards prescribed by § 440.19a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The sodium dicloxacillin monohydrate used in making the batch for potency, moisture, pH, organic chlorine content, free chloride content, crystallinity, and identity.

(*b*) The batch for potency, sterility, pyrogens, safety, moisture, and pH.

(ii) Samples required:

(*a*) The sodium dicloxacillin monohydrate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 15 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Reconstitute as directed in the labeling. Using a suitable hypodermic needle and syringe remove all of the withdrawable contents if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the sample thus obtained with sufficient 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), for the microbiological agar diffusion assay or in distilled water for the iodometric assay and hydroxylamine colorimetric assay, to give a stock solution of convenient concentration.

(ii) *Assay procedure.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 5 micrograms of dicloxacillin per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, diluting an aliquot of the stock solution with distilled water to the prescribed concentration.

(*c*) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter, except dilute an aliquot of the stock solution with distilled water to the prescribed concentration.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32 (a) of this chapter, using a solution containing 20 milligrams of dicloxacillin per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this chapter, except use a test dose of 0.5 milliliter of a solution containing 4.0 milligrams of dicloxacillin.

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using the product reconstituted as directed in the labeling.

§ 440.229 Potassium hetacillin injectable dosage forms.

§ 440.229a Sterile potassium hetacillin.

The requirements for certification and the tests and methods of assay for sterile potassium hetacillin packaged for dispensing are described in § 440.29a.

§ 440.229b Potassium hetacillin for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Potassium hetacillin for injection is a dry mixture of potassium hetacillin and lidocaine hydrochloride. Its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of ampicillin that it is represented to contain. It is sterile, nonpyrogenic, and passes the safety test. Its moisture content is not more than 1.0 percent. When reconstituted as directed in its labeling, its pH is not less than 7.0 and not more than 9.0. The potassium hetacillin used conforms to the requirements of § 440.29a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The potassium hetacillin used in making the batch for potency, moisture, pH, potassium hetacillin content, identity, and crystallinity.

(*b*) The batch for potency, sterility, pyrogens, safety, moisture and pH.

(ii) Samples required:

(*a*) The potassium hetacillin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers, except if each contains less than 450 milligrams of ampicillin, a minimum of 16 immediate containers.

(2) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed for ampicillin in § 436.105 of this chapter, using the ampicillin working standard as the standard of comparison and preparing the sample for assay as follows: Reconstitute as directed in the labeling. Using a suitable hypodermic needle and syringe, remove the withdrawable contents from each container represented as a single-dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, withdraw an accurately measured representative portion from each container. Dilute the sample thus obtained with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute the stock solution with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing the equivalent of 18 milligrams of ampicillin per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this chapter for potassium hetacillin, except use a concentration of 4 milligrams of ampicillin activity per milliliter.

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using the product reconstituted as directed in the labeling.

§ 440.236 Sodium methicillin for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium methicillin for injection is sodium methicillin either containing ethylenediaminetetraacetic acid trisodium salt in a quantity not less than 4.25 percent and not more than 5.25 percent by weight of its total solids or containing a suitable preservative and the buffer sodium citrate in a quantity not less than 4 percent and not more than 5 percent by weight of its total solids (such sodium citrate conforms to the standards prescribed therefor by the U.S.P.). Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of methicillin that it is represented to contain. It is sterile. It is nonpyrogenic. It passes the safety test. If it contains ethylenediaminetetraacetic acid trisodium salt, its pH, immediately after reconstitution as directed in the labeling, is not less than 7.0 and not more than 8.5; if it contains sodium citrate buffer, its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 6.0 and not more than 8.5. Its moisture content is not more than 6.0 percent. The sodium methicillin used conforms to the standards prescribed by § 440.36a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The sodium methicillin used in making the batch for potency, moisture, pH, sodium methicillin content, crystallinity, and identity.

(*b*) The batch for potency, sterility, pyrogens, safety, pH, and moisture.

(ii) Samples required:

(*a*) The sodium methicillin used in making the batch: 10 packages, each containing approximately 300 milligrams,

plus one package containing approximately 2 grams.

(b) The batch:

(1) For all tests except sterility: A minimum of 10 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Reconstitute as directed in the labeling. Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the portion thus obtained with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration.

(ii) *Assay procedure.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(a) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this subchapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 10 micrograms of methicillin per milliliter (estimated).

(b) *Iodometric assay.* Proceed as directed in § 436.204 of this subchapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(2) *Sterility.* Proceed as directed in § 436.20 of this subchapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this subchapter, using a solution containing 20 milligrams of methicillin per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this subchapter, except use a test dose solution containing 40 milligrams of methicillin per milliliter.

(5) *pH.* Proceed as directed in § 436.202 of this subchapter, using the drug reconstituted as directed in the labeling if it contains ethylenediaminetetraacetic acid trisodium salt or using an aqueous solution containing 10 milligrams per milliliter if it contains sodium citrate buffer.

(6) *Moisture.* Proceed as directed in § 436.201 of this subchapter.

§ 440.241 Sodium nafcillin monohydrate for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium nafcillin monohydrate for injection is a dry mixture of sodium nafcillin monohydrate and a suitable buffer substance. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of nafcillin that it is represented to contain. It is sterile. It is nonpyrogenic. It passes the safety test. Its moisture content is not less than 3.5 and not more than 5.3 percent. When reconstituted as directed in the labeling, the pH is not less than 5.0 and not more than 8.0. The sodium nafcillin monohydrate used conforms to the requirements of § 440.41a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The sodium nafcillin monohydrate used in making the batch for potency, moisture, pH, crystallinity, sodium nafcillin monohydrate content, and identity.

(b) The batch for potency, sterility, pyrogens, safety, moisture, and pH.

(ii) Samples required:

(a) The sodium nafcillin monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 12 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Reconstitute as directed in the labeling. Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container, or if the labeling specifies the amount of potency in a given volume of the resultant preparation remove an accurately measured representative portion from each container. Dilute the sample thus obtained with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 2.0 micrograms of nafcillin per milliliter (estimated) for the microbiological agar diffusion assay and to the prescribed concentration for the iodometric assay.

(ii) *Assay procedures.* Assay for potency by either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(a) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 this chapter.

(b) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 20 milligrams of nafcillin per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this chapter.

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using the solution obtained when the product is reconstituted as directed in the labeling.

§ 440.249 Sodium oxacillin for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium oxacillin for injection is a dry mixture of sodium oxacillin and one or more buffer substances, with or without trisodium ethylenediamine tetraacetic acid, and with or without one or more suitable and harmless preservatives. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of oxacillin that it is represented to contain. It is sterile. It is nonpyrogenic. It passes the safety test. Its moisture content is not more than 6.0 percent. Its pH in an aqueous solution containing 30 milligrams per milliliter is not less than 6.0 and not more than 8.5. The sodium oxacillin used conforms to the standards prescribed by § 440.49a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The sodium oxacillin used in making the batch for potency, moisture, pH, sodium oxacillin content, crystallinity, and identity.

(b) The batch for potency, sterility, pyrogens, safety, moisture, and pH.

(ii) Samples required:

(a) The sodium oxacillin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 10 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation, or 40 immediate containers if each contains less than 600 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container, or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration.

(ii) *Assay procedures.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(a) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 5 micrograms of oxacillin per milliliter (estimated).

(b) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 20 milligrams of oxacillin per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this chapter.

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 30 milligrams per milliliter.

§ 440.253 Aluminum penicillin in oil.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality and purity.* Aluminum penicillin in oil is a suspension of aluminum penicillin in a menstruum of refined peanut oil or sesame oil. Its potency is 300,000 units per milliliter. Its moisture content is not more than 1.5 percent. It is sterile. The aluminum penicillin used conforms to the requirements of § 440.53a(a)(1). The peanut oil or sesame oil used conforms to the standards prescribed therefor by the U.S.P.

(2) *Packaging.* The immediate container of aluminum penicillin in oil shall be of colorless, transparent glass, so closed as to be a tight container as defined by the U.S.P., shall be sterile at the time of filling and closing, shall be so sealed that its contents cannot be used without destroying such seal, and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded. The quantity of aluminum penicillin in oil in each such container shall be not less than 1 milliliter and not more than 20 milliliters, unless it is packaged for repacking. Unless it is packaged for repacking, each container shall be filled with a volume of aluminum penicillin in oil in excess of that designated, which excess shall be sufficient to permit the withdrawal and the administration of the volume indicated, whether administered in either single or multiple doses.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the outside wrapper or container and the immediate container, the statement "Expiration date _____", the blank being filled in with the date that is 12 months after the month during which the batch was certified.

(ii) On the outside wrapper or container, the statement "Store in refrigerator not above 15° C. (59° F.)".

(4) *Requests for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch of aluminum penicillin in oil shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless it was previously submitted) the date on which the latest assay of the aluminum penicillin used in making such batch was completed, the number of units in each of such packages, the quantity of each ingredient used in making the batch, the date on which the latest assay of the drug comprising such batch was completed, and that the peanut oil or sesame oil used in making such batch conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request results of the tests and assays listed after each of the following, made by him on an accurately representative sample of:

(*a*) The batch; potency, sterility, moisture.

(*b*) The aluminum penicillin used in making the batch; potency, sterility, toxicity, pyrogens, moisture, pH, penicillin K content.

(iii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch:

(*1*) For all tests except sterility: One package for each 500 packages in the batch, but in no case less than three packages or more than 12 packages.

Such samples shall be collected by taking single packages at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(*2*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation when each contains not less than 600,000 units or not less than 2.0 milliliters, or 40 immediate containers when each contains less than these amounts.

(*b*) The aluminum penicillin used in making the batch:

(*1*) For all tests except sterility: Six packages, each containing approximately equal portions of not less than 300 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 600 milligrams.

Each such portion shall be taken from a different part of such batch, and each shall be packaged in accordance with the requirements of § 440.53a(a)(2).

(*c*) In case of an initial request for certification, the peanut oil or sesame oil used in making the batch; one package of each containing approximately 250 grams.

(iv) No result referred to in paragraph (a)(4)(ii)(*b*) of this section, and no sample referred to in paragraph (a)(4)(iii)(*b*) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay—*(1) *Potency, sterility.* Proceed as directed in § 436.500.

(2) *Moisture.* Proceed as directed in § 440.280d(b)(3).

§ 440.255 Benzathine penicillin G injectable dosage forms.

§ 440.255a Benzathine penicillin G in oil.

(a) *Requirements for certification.* Benzathine penicillin G in oil conforms to all requirements and is subject to all procedures prescribed by § 440.274a(a) for sterile procaine penicillin in oil, except that:

(1) Benzathine penicillin G is used in lieu of procaine penicillin. The benzathine penicillin used conforms to the requirements of § 440.55a(a)(1).

(2) Its moisture content is not more than 4 percent.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 440.80a(b)(1), except paragraph (b)(1)(ix) of that section, and in lieu of the directions in § 440.80a(b)(1)(iv) prepare the sample as follows: Place a representative quantity (usually 1.0 milliliter) of a multiple-dose container, or the entire contents of a single-dose container, in a blending jar containing sufficient dimethyl formamide (previously adjusted to a pH of 6.5±0.5 with concentrated H_2SO_4) or redistilled methanol to give a final volume of 100 milliliters. Using a high-speed blender, blend the mixture for 2 minutes and then make the proper estimated dilutions in a 1.0-percent phosphate buffer at pH 6.0. The potency is satisfactory if it contains not less than 85 percent of the number of units that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter using the method described in paragraph (e)(2) of that section, except use medium C in lieu of medium A, and medium F in lieu of medium E. During the period of incubation, shake the tubes at least once daily.

(3) *Moisture.* Proceed as directed in § 436.500(c).

§ 440.255b Benzathine penicillin G for aqueous injection.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Benzathine penicillin G for aqueous injection is a dry mixture of benzathine penicillin G and one or more suitable and harmless suspending or dispersing agents and with or without one or more suitable and harmless preservatives, buffer substances, and local anesthetics; or it is an aqueous suspenion of benzathine penicillin G and one or more suitable and harmless suspending or dispersing agents, buffer substances, and preservatives and with or without one or more suitable and harmless local anesthetics. It is so purified that:

(i) If it is an aqueous suspension of the drug, each container or each milliliter shall contain not less than 300,000 units.

(ii) It is sterile.

(iii) If it is the dry mixture of the drug, its moisture content is not more than 8 percent.

(iv) It is nonpryogenic.

(v) It is nontoxic.

(vi) Its pH in saturated solution is not less than 5.0 and not more than 7.5.

The benzathine penicillin G used conforms to the requirements of § 440.55a (a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* In all cases the immediate containers shall be tight containers as defined by the U.S.P., shall be sterile at the time of filling and closing, shall be so sealed that the contents cannot be used without destroying such seal, and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded. In case it is packaged for dispensing, it shall be in immediate containers of colorless transparent glass, closed by a substance through which a hypodermic needle may be introduced and withdrawn without removing the closure or destroying its effectiveness. If it is the dry mixture of the drug, each such container shall contain 300,000 units, 600,000 units, 900,000 units, 1,200,000 units, 1,500,000 units, 2,400,000 units, or 3,000,000 units, unless it is intended solely for veterinary use and is conspicuously so labeled. Each such container may be packaged in combination with a container of a suitable aqueous diluent. If it is the aqueous suspension of the drug, each such container shall contain not less than 1 milliliter (unless it is packaged to contain a single dose) and not more than 10 milliliters, and each shall be filled with a volume in excess of that designated, which excess shall be sufficient to permit the withdrawal and the administration of the volume indicated, whether administered in either single or multiple doses.

(3) *Labeling.* In addition to the requirements of § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its label and labeling, as hereinafter indicated, the following:

(i) On the outside wrapper or container and the immediate container:

(*a*) The statement "Expiration date ----------", the blank being filled in, if it is a dry mixture of the drug, with the date that is 48 months, or if it is the aqueous suspension of the drug, with the date that is 18 month after the month during which the batch was certified, except that the blank may be filled in with the date that is 24 months, 36 months, 48 months, or 60 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time such drug as prepared by him complies with the standards prescribed by paragraph (a)(1) of this section.

(*b*) If it is the aqueous suspension of the drug the statement "Store in refrigerator not above 15° C. (59° F.)" or "Store below 15° C. (59° F.)", unless the person who requests certification has submitted to the Commissioner results of tests and assays showing that such drug as prepared by him complies with the standards prescribed by paragraph (a)(1) of this section after having been stored at room temperature.

(ii) On the circular or other labeling within or attached to the package, if it is the dry mixture of the drug, the conditions under which suspensions made from such drug should be stored, and the statement "Sterile suspension may be kept at room temperature for 1 week, or in refrigerator for 3 weeks, without significant loss of potency."

(4) *Request for certification; samples.* (i) In addition to complying with the requirement of § 431.1 of this chapter, a person who requests certification of a batch of benzathine penicillin G for aqueous injection shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless it was previously submitted) the date on which the latest assay of the benzathine penicillin G used in making such batch was completed, the number of units in each of such packages, the quantity of each ingredient used in making the batch, the date on which the latest assay of the drug comprising such batch was completed, and a statement that each ingredient used in making the batch conforms to the requirements prescribed therefor, if any, by this section. If such batch, or any part thereof, is to be packaged with a solvent, such request shall also be accompanied by a statement that such solvent conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided by paragraph (a)(4)(v) of this section, such person shall submit in connection with his request results of the tests and assays listed after each of the following, made by him on an accurately representative sample of:

(*a*) The batch; potency, sterility, moisture (unless it is an aqueous suspension of the drug), pyrogens, toxicity, pH.

(*b*) The benzathine penicilin G used in making the batch; potency, penicillin G content, crystallinity.

(iii) Except as otherwise provided by paragraph (a)(4)(v) of this section, if such batch is packaged for dispensing, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch:

(*1*) For all tests except sterility: One immediate container for each 5,000 immediate containers in such batch, but in no case less than 10 immediate containers.

Such samples shall be collected by taking single immediate containers at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation, or 40 immediate containers if each contains less than 600 milligrams.

(*b*) The benzathine penicillin G used in making the batch; three packages containing approximately equal portions of not less than 500 milligrams each, packaged in accordance with the requirements of § 440.55a(a)(1).

(*c*) In case of an initial request for certification, each other ingredient used in making the batch; one package of each containing approximately 5 grams.

(*d*) In case of an initial request for the certification of a batch of benzathine penicillin G for aqueous injection which is to be packaged in combination with an aqueous diluent which is not recognized by the U. S. P., or when any change is made in the composition of such diluent, five packages of the diluent included in the combination.

(iv) If such batch is packaged for repacking, such person shall submit with his request a sample consisting of the following:

(*a*) For all tests except sterility: 10 packages; each containing approximately 300 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 600 milligrams.

Each such package shall be taken from a different portion of such batch, and each shall be packaged in accordance with the requirements of paragraph (a)(2) of this section.

(v) No result referred to in paragraph (a)(4)(ii)(*b*) of this section, and no sample referred to in paragraph (a)(4)(iii)(*b*) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 440.55a (b)(1), using as the sample for assay a representative aliquot of the suspension equivalent to one dose; or if it is a dry mixture of the drug, a representative aliquot of the drug equivalent to one dose after it has been reconstituted as directed in the labeling. Its potency is satisfactory if it contains not less than 90 percent of the number of units that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except use medium C in lieu of medium A, and medium F in lieu of medium E. During the period of incubation, shake the tubes at least once daily.

(3) *Moisture (dry mixture of the drug).* Proceed as directed in § 440.74a (b)(5).

(4) *Pyrogens.* Proceed as directed in § 440.55a(b)(4).

(5) *Toxicity.* Proceed as directed in § 440.55a(b)(3).

(6) *pH*—(i) *Dry mixture of the drug.* Proceed as directed in § 440.80a(b)(5)(ii) using the suspension resulting when the product is reconstituted as directed in the labeling.

(ii) *Aqueous suspension of the drug.* Proceed as directed in §440.80a(b)(5)(ii), using the undiluted aqueous suspension.

§ 440.255e Benzathine penicillin G and procaine penicillin for aqueous injection.

(a) *Requirements for certification.* Benzathine penicillin G and procaine penicillin for aqueous injection conforms to all requirements, and is subject to all procedures, prescribed by § 440.255b(a) for benzathine penicillin G for aqueous injection, except that:

(1) Each container shall contain not less than 300,000 units of benzathine penicillin G and not less than 300,000 units of procaine penicillin. The procaine penicillin used conforms to the requirements prescribed therefor by § 440.74a(a)(1).

(2) If it is the dry mixture of the drug, its moisture content is not more than 6.0 percent.

(3) In lieu of the directions for labeling prescribed by § 440.255b(a)(3)(i)(*a*)(1), each package shall bear on the outside wrapper or container and the immediate container the statement, "Expiration date _____", the blank being filled in, if it is the aqueous suspension of the drug, with the date that is 18 months after the month during which the batch was certified, except that the blank may be filled in with the date that is 24 months if the person requesting certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time such drug as prepared by him complies with the standards prescribed for it, or if it is the dry mixture of the drug, with the date that is 36 months after the month during which the batch was certified, except that the blank may be filled in with the date that is 48 months or 60 months if the person requesting certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time such drug as prepared by him complies with the standards prescribed for it.

(4) In addition to complying with the requirements of § 440.255b(a)(4), a person who requests certification of a batch of benzathine penicillin G and procaine penicillin for aqueous injection shall submit with his request a statement showing the batch mark, and (unless it was previously submitted) the results and the date of the latest tests and assays of the procaine penicillin used in making the batch for potency, crystallinity, penicillin K content (unless it is procaine penicillin G) and the penicillin G content if it is procaine penicillin G, and the number of units of procaine penicillin in each milliliter of the batch, if it is the aqueous suspension of the drug, or the number of units of procaine penicillin in each package of the batch, if it is the dry mixture of the drug. He shall also submit in connection with his request a sample consisting of three packages containing approximately equal portions of not less than 500 milligrams each of the procaine penicillin used in making the batch. If such batch is packaged for repacking, each portion in the sample required by § 440.255b(a)(4)(iv)(*a*) shall consist of the equivalent of approximately 600 milligrams in lieu of 300 milligrams.

(b) *Tests and methods of assay*—(1) *Total potency, benzathine penicillin G content, procaine penicillin content.*

(i) If the product does not contain procaine hydrochloride: Proceed as directed in § 436.507(a) (1), (2), and (4) of this chapter.

(ii) If the product contains procaine hydrochloride: (*a*) *Total potency.* Place one dose of the suspension in each of two centrifuge tubes. Label one tube "sample" and the other "blank". Add 9.0 ml. of 20 percent sodium sulfate solution to each tube and mix well. Centrifuge them at 10,000 r.p.m. for 15 minutes or until a clear supernatant solution is obtained. Pour the supernatant solution from the sample tube into a 50-ml. volumetric flask and dilute to mark with 1 percent pH 6.0 buffer. Determine the amount of pencillin in this flask by the iodometric assay procedure. Wash the penicillin residue from the tube labeled "sample" into a 200-ml. volumetric flask quantitatively with about 150 ml. of water. Add 10 ml. of 1.0*N* sodium hydroxide and allow to stand for 15 minutes. Acidify the solution with 10 ml. of 1.2*N* hydrochloric acid and dilute to mark with water Determine the amount of penicillin in this solution by the iodometric assay procedure. Use as a blank a suspension prepared by discarding the supernatant solution from the tube labeled "blank", washing the penicillin residue into a 200-ml. volumetric flask, and making to mark with water. The total potency of the dose is the sum of the penicillin in the sample supernatant solution and in the insoluble residue of the sample.

(*b*) *Procaine penicillin content.* Transfer 5.0 ml. of the solution from the 200-ml. volumetric flask containing the residue from the "sample" tube to a 50-ml. volumetric flask and make to mark with water. Assay 2.0 ml. of the solution by the Bratton-Marshall method. The amount of procaine penicillin is the sum of the amount of penicillin in the sodium sulfate supernatant solution and the amount of penicillin determined by the Bratton-Marshall method.

(*c*) *Benzathine penicillin content.* The amount of benzathine penicillin is the difference between the total potency and the amount of procaine penicillin.

(2) *Sterility, toxicity, pyrogens.* Proceed as directed in § 440.55a(b) (2), (3), and (4).

(3) *pH.* Proceed as directed in § 440.80a(b)(5)(ii), using the undiluted aqueous suspension or the suspension prepared as directed in the labeling for the drug.

(4) *Moisture (dry mixture of the drug).* Proceed as directed in § 440.74a(b)(5).

§ 440.259 Chloroprocaine penicillin O for aqueous injection.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Chloroprocaine penicillin O for aqueous injection is a dry mixture of chloroprocaine penicillin O and one or more suitable and harmless suspending or dispersing agents, with or without one or more suitable and harmless buffer substances. It is so purified and dried that:

(i) It is sterile.

(ii) Its moisture content is not more than 4.2 percent.

(iii) It is nonpyrogenic.

(iv) It is nontoxic.

(v) Its pH in saturated aqueous solution is not less than 5.0 and not more than 7.5.

The chloroprocaine penicillin O used conforms to the requirements of § 440.59a(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* In all cases the immediate containers shall be tight containers as defined by the U. S. P., shall be sterile at the time of filling and closing, shall be so sealed that the contents cannot be used without destroying such seal, and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded. In case it is packaged for dispensing, it shall be in immediate containers of colorless, transparent glass, closed by a substance through which a hypodermic needle may be introduced and withdrawn without removing the closure or destroying its effectiveness. Each such container shall contain 300,000 units, 600,000 units, 900,000 units, 1,200,000 units 1,500,000 units, or 3,000,000 units unless it is intended solely for veterinary use and is conspicuously so labeled. Each such container may be packaged in combination with a container of a suitable aqueous diluent.

(3) *Labeling.* In addition to the requirements of § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its label and labeling as hereinafter indicated, the following:

(i) On the outside wrapper or container and the immediate container, the statement "Expiration date _____", the blank being filled in with the date that is 36 months after the month during which the batch was certified.

(ii) On the circular or other labeling within or attached to the package, the and the statement "Sterile suspension conditions under which suspensions made from such drug should be stored, may be kept at room temperature for 1 week, or in refrigerator for 3 weeks, without significant loss of potency".

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch of chloroprocaine penicillin O for aqueous injection shall

submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless it was previously submitted) the date on which the latest assay of the chloroprocaine penicillin O used in making such batch was completed, the number of units in each of such packages, the quantity of each ingredient used in making the batch, the date on which the latest assay of the drug comprising such batch was completed, and a statement that each ingredient used in making the batch conforms to the requirements prescribed therefor, if any, by this section. If such batch, or any part thereof, is to be packaged with a solvent, such request shall also be accompanied by a statement that such solvent conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided by paragraph (a)(4)(v) of this section, such person shall submit in connection with his request results of the tests and assays listed after each of the following, made by him on an accurately representative sample of:

(*a*) The batch; potency, sterility, moisture, pyrogens, toxicity, pH.

(*b*) The chloroprocaine penicillin O used in making the batch; potency crystallinity, penicillin O content, and penicillin G content.

(iii) Except as otherwise provided by paragraph (a)(4)(v) of this section, if such batch is packaged for dispensing, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(a) The batch;

(*1*) For all tests except sterility: One immediate container for each 5,000 immediate containers in such batch, but in no case less than 10 immediate containers.

Such samples shall be collected by taking single immediate containers at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(2) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation, or 40 immediate containers if each contains less than 600 milligrams.

(*b*) The chloroprocaine penicillin O used in making the batch: six packages containing approximately equal portions of not less than 300 milligrams each.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch; one pacakage of each containing approximately 5.0 grams.

(*d*) In case of an initial request for the certification of a batch of chloroprocaine penicillin O for aqueous injection which is to be packaged in combination with an aqueous diluent which is not recognized by the U.S.P., or when any change is made in the composition of such diluent, five packages of the diluent included in the combination.

(iv) If such batch is packaged for repacking, such person shall submit with his request a sample consisting of the following:

(*a*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*b*) For sterility testing: 20 packages each containing approximately 600 milligrams.

Each such package shall be taken from a different part of such batch, and each shall be packaged in accordance with the requirements of paragraph (a)(2) of this section.

(v) No result referred to in paragraph (a)(4)(ii)(*b*) of this section, and no sample referred to in paragraph (a)(4)(iii)(*b*) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay.* For the determination of potency, sterility, moisture, pyrogens, toxicity, and pH, proceed as directed in § 440.274b (b), except if the iodometric assay for potency is used, calculate as directed in § 440.80a(b)(5)(iv)(*b*).

§ 440.261 Diethylaminoethyl ester penicillin G hydriodide for aqueous injection (penicillin G diethylaminoethyl ester hydriodide for aqueous injection).

(a) *Requirements for certification—*

(1) *Standards of identity, strength, quality, and purity.* Diethylaminoethyl ester penicillin G hydriodide for aqueous injection is diethylaminoethyl ester penicillin G hydriodide and one or more suitable and harmless suspending or dispersing agents, with or without one or more suitable and harmless preservatives and buffer substances. It is so purified and dried that:

(i) It is sterile.

(ii) Its moisture content is not more than 1 percent.

(iii) It is nonpyrogenic.

(iv) It is nontoxic.

(v) The pH of a saturated aqueous solution is not less than 5.0 and not more than 7.5.

The diethylaminoethyl ester penicillin G hydriodide used conforms to the requirements of § 440.61a(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* In all cases the immediate containers shall be tight containers as defined by the U. S. P., shall be sterile at the time of filling and closing, shall be so sealed that the contents cannot be used without destroying such seal, and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded. In case it is packaged for dispensing, it shall be in immediate containers of colorless transparent glass, closed by a substance through which a hypodermic needle may be introduced and withdrawn without removing the closure or destroying its effectiveness. Each such container shall contain 500,000 units or multiples thereof up to and including 5,000,000 units, and each may be packaged in combination with a container of a suitable aqueous diluent.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the outside wrapper or container and the immediate container, the statement "Expiration date _____", the blank being filled in with the date that is 24 months after the month during which the batch was certified.

(ii) On the circular or other labeling within or attached to the package, the conditions under which suspensions of the drug may be stored, and the statement "Sterile suspension may be kept at room temperature for 7 days, or in refrigerator for 3 weeks, without significant loss of potency".

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless it was previously submitted) the date on which the latest assay of the diethylaminoethyl ester penicillin G hydriodide used in making such batch was completed, the number of units in each such packages, the quantity of each ingredient used in making the batch, the date on which the latest assay of the drug comprising such batch was completed, and a statement that each ingredient used in making the batch conforms to the requirements prescribed therefor by this section. If such batch, or any part thereof, is to be packaged with a solvent, such request shall be accompanied by a statement that such solvent conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided by paragraph (a)(4)(v) of this section, such person shall submit in connection with his request results of the tests and assays listed after each of the following, made by him on an accurately representative sample of:

(*a*) The batch; potency, sterility moisture, pyrogens, toxicity, pH.

(*b*) The diethylaminoethyl ester penicillin G hydriodide used in making the batch; potency, crystallinity, penicillin G content.

(iii) Except as otherwise provided by paragraph (a)(4)(v) of this section, if such batch is packaged for dispensing such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch:

(*1*) For all tests except sterility: One immediate container for each 5,000 im-

mediate containers in such batch, but in no case less than 10 immediate containers.

Such samples shall be collected by taking single immediate containers at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation, or 40 immediate containers if each contains less than 600 milligrams.

(*b*) The diethylaminoethyl ester penicillin G hydriodide used in making the batch; 3 packages containing approximately equal portions of not less than 500 milligrams each, packaged in accordance with the requirements of § 440.61a(a)(2).

(*c*) In case of an initial request for certification, each other ingredient used in making the batch; one package of each containing approximately 5 grams.

(*d*) In the case of an initial request for the certification of a batch which is to be packaged in combination with an aqueous diluent which is not recognized by the U.S.P., or when any change is made in he composition of such diluent; 5 packages of the diluent included in the combination.

(iv) If such batch is packaged for repacking, such person shall submit with his request a sample consisting of the following:

(*a*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 600 milligrams.

Each such package shall be taken from a different portion of such batch, and each shall be packaged in accordance with the requirements of paragraph (a)(2) of this section.

(v) No result referred to in paragraph (a)(5)(ii)(*b*) of this section, and no sample referred to in paragraph (a)(4)(iii)(*b*) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay*—(1) *Potency.* Using a representative aliquot of the product reconstituted as directed in the labeling, proceed as directed in § 440.61a(b)(1). Its potency is satisfactory if it contains not less than 90 percent of the number of units that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except add sufficient penicillinase either to diluting fluid A or, if the product contains lecithin, to diluting fluid D, and swirl the flask to completely solubilize the preparation.

(3) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i).

(4) *Pyrogens.* Proceed as directed in § 440.80a(b)(3), except use physiological salt solution as the diluent.

(5) *Toxicity.* Proceed as directed in § 440.80a(b)(4), except use physiological salt solution as the diluent, and inject 0.5 milliliter of a solution containing 2,000 units per milliliter.

(6) *pH.* Proceed as directed in § 440.80a(b)(5)(ii), using the suspension resulting when the product is reconstituted as directed in the labeling.

§ 440.265 *l*-Ephenamine penicillin G injectable dosage forms.

§ 440.265a *l*-Ephenamine penicillin G in oil.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* *l*-Ephenamine penicillin G in oil is a suspension of *l*-ephenamine penicillin G in refined peanut oil or sesame oil with or without the addition of one or more suitable and harmless dispersing agents. Its potency is 300,000 units per milliliter unless it is packaged and labeled solely for veterinary use. It is sterile. Its moisture content is not more than 1 percent. The *l*-ephenamine penicillin G used conforms to the requirements of § 440.65a(a)(1). Each other substance used, if its name is recognized in the U. S. P. or N. F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* The immediate container shall be of colorless transparent glass (unless it is packaged to contain a single dose), so closed as to be a tight container as defined by the U. S. P., shall be sterile at the time of filling and closing, and shall be so sealed that the contents cannot be used without destroying such seal. The immediate container shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded. If it is packaged for dispensing, each such container shall contain not less than 1.0 milliliter and not more than 20 milliliters and each shall be filled with a volume in excess of that designated, which excess shall be sufficient to permit the withdrawal and the administration of the volume indicated, whether administered in single or multiple doses.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act) each package shall bear on the outside wrapper or container and the immediate container the statement "Expiration date _____", the blank being filled in with the date that is 18 months after the month during which the batch was certified.

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless it was previously submitted) the date on which the latest assay of the *l*-ephenamine penicillin G used in making such batch was completed, the number of units in each of such packages, the quantity of each ingredient used in making the batch, the date on which the latest assay of the drug comprising such batch was completed, and that each ingredient used in making such batch conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided by paragraph (a)(4)(iv) of this section such person shall submit in connection with his request results of tests and assays of the following made by him on an accurately representative sample of:

(*a*) The batch; potency, sterility, and moisture.

(*b*) The *l*-ephenamine penicillin G used in making the batch; potency, sterility, pyrogens, toxicity, moisture, pH, crystallinity, heat stability, penicillin G content, and specific rotation.

(iii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch:

(*1*) For all tests except sterility: One package for each 5,000 packages in the batch, but in no case less than five packages.

Such samples shall be collected by taking single packages at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(2) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation when each container contains not less than 600,000 units or not less than 2.0 milliliters, or 40 immediate containers when each contains less than these amounts.

(*b*) The *l*-ephenamine penicillin G used in making the batch:

(*1*) For all tests except sterility: 10 packages, each containing approximately equal portions of not less than 300 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 600 milligrams.

Each such portion shall be taken from a different part of such batch and each shall be packaged in accordance with the requirements of § 440.65a(a)(2).

(*c*) In case of an initial request for certification, one package containing approximately 150 grams of the peanut oil or sesame oil used and one package containing approximately 5 grams of each dispersing agent used.

(iv) No result referred to in paragraph (a)(4)(ii)(*b*) of this section, and no sample referred to in paragraph (a)(4)(iii)(*b*) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 440.274a(b)(1), except the last sentence thereof. If it is represented to contain less than 300,000 units per milliliter, its potency is satisfactory if it contains not less than 85 percent of the number of units so represented. If it is represented to contain 300,000 units per milliliter, its potency is

satisfactory if it contains not less than 90 percent of the number of units so represented.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except use medium C in lieu of medium A, and medium F in lieu of medium E.

(3) *Moisture.* Proceed as directed in § 436.500(c) of this chapter.

§ 440.265b *l*-Ephenamine penicillin G for aqueous injection.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* *l*-Ephenamine penicillin G for aqueous injection is a dry mixture of *l*-ephenamine pencillin G and one or more suitable and harmless suspending or dispersing agents, with or without one or more suitable and harmless preservatives and buffer substances, or it is an aqueous suspension of *l*-ephenamine penicillin G and one or more suitable and harmless suspending or dispersing agents, buffer substances, and preservatives, except that preservatives are not required if the immediate container is packaged to contain a single dose and is conspicuously so labeled. It is so purified that:

(i) If it is an aqueous suspension of the drug, each container or each milliliter shall contain not less than 300,000 units, unless it is packaged and labeled solely for veterinary use;

(ii) It is sterile;

(iii) If it is the dry mixture of the drug, its moisture content is not more than 1.5 percent;

(iv) It is nonpyrogenic;

(v) It is nontoxic; and

(vi) Its pH in saturated aqueous solution is not less than 5.0 and not more than 7.5.

The *l*-ephenamine penicillin G used conforms to the requirements of § 440.65a(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* In all cases the immediate containers shall be tight containers as defined by the U.S.P., shall be sterile at the time of filling and closing, shall be so sealed that the contents cannot be used wihout destroying such seal, and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded. In case it is packaged for dispensing, it shall be in immediate containers of colorless transparent glass, closed by a substance through which a hypodermic needle may be introduced and withdrawn without removing the closure or destroying its effectiveness, unless it is the aqueous suspension of the drug and it is packaged to contain a single dose. If it is the dry mixture of the drug, each such container shall contain 300,000 units, 600,000 units, 900,000 units, 1,200,000 units, 1,500,000 units or 3,000,000 units (unless it is packaged and labeled solely for veterinary use), and each may be packaged in combination with a container of a suitable aqueous diluent. If it is the aqueous suspension of the drug, each such container shall contain not less than 1 milliliter (unless it is packaged to contain a single dose) and not more than 10 milliliters (unless it is packaged and labeled solely for veterinary use), and each shall be filled with a volume in excess of that designated, which excess shall be sufficient to permit the withdrawal and the administration of the volume indicated, whether administered in either single or multiple doses.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the outside wrapper or container and the immediate container, the statement "Expiration date _____", the blank being filled in, if it is the dry mixture of the drug, with the date that is 18 months, or if it is the aqueous suspension of the drug, with the date that is 12 months after the month during which the batch was certified, except that the blank may be filled in with the date that is 24 months, 36 months, 48 months, or 60 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time such drug as prepared by him complies with the standards prescribed by paragraph (a)(1) of this section.

(ii) On the outside wrapper or container, if it is the aqueous suspension of the drug, the statement "Store in refrigerator not above 15° C. (59° F.)" or "Store below 15° C. (59° F.)", unless the person who requests certification has submitted to the Commissioner results of tests and assays showing that such drug as prepared by him complies with the standards prescribed by paragraph (a)(1) of this section after having been stored at room temperature.

(iii) On the circular or other labeling within or attached to the package, if it is the dry mixture of the drug and is packaged for dispensing, the conditions under which suspensions made from such drug should be stored, and the statement "Sterile suspension may be kept at room temperature for 1 week or in refrigerator for 3 weeks, without significant loss of potency".

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch of *l*-ephenamine penicillin G for aqueous injection shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless it was previously submitted) the date on which the latest assay of the *l*-ephenamine penicillin G used in making such batch was completed, the number of units in each of such packages, the quantity of each ingredient used in making the batch, the date on which the latest assay of the drug comprising such batch was completed, and a statement that each ingredient used in making the batch conforms to the requirements prescribed therefor by this section. If such batch, or any part thereof, is to be packaged with a solvent, such request shall also be accompanied by a statement that such solvent conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided by paragraph (a)(4)(v) of this section, such person shall submit in connection with his request results of the tests and assays listed after each of the following, made by him on an accurately representative sample of:

(*a*) The batch; potency, sterility moisture (unless it is the aqueous suspension of the drug), pyrogens, toxicity, pH.

(*b*) The *l*-ephenamine penicillin G used in making the batch; potency, crystallinity, heat stability, penicillin G content, and specific rotation.

(iii) Except as otherwise provided by paragraph (a)(4)(v) of this section, if such batch is packaged for dispensing, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch:

(*1*) For all tests except sterility: One immediate container for each 5,000 immediate containers in such batch, but in no case less than 10 immediate containers.

Such samples shall be collected by taking single immediate containers at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(*2*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation, or 40 immediate containers if each contains less than 600 milligrams.

(*b*) The *l*-ephenamine penicillin G used in making the batch; 3 packages containing approximately equal portions of not less than 500 milligrams each, packaged in accordance with the requirements of § 440.65a(a)(2).

(*c*) In case of an initial request for certification, each other ingredient used in making the batch, one package of each containing approximately 5 grams.

(*d*) In case of an initial request for the certification of a batch which is to be packaged in combination with an aqueous diluent which is not recognized by the U.S.P., or when any change is made in the composition of such diluent; 5 packages of the diluent included in the combination.

(iv) If such batch is packaged for repacking, such person shall submit with his request a sample consisting of the following:

(*a*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 600 milligrams.

Each such package shall be taken from a different part of such batch, and each shall be packaged in accordance with the requirements of paragraph (a) (2) of this section.

(v) No result referred to in paragraph (a) (4) (ii) (*b*) of this section, and no sample referred to in paragraph (a) (4) (iii) (*b*) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 440.65a (b) (1), using as the sample for assay a representative aliquot of the suspension equivalent to one dose; or if it is a dry mixture of the drug, a representative aliquot of the drug equivalent to one dose after it has been reconstituted as directed in the labeling. Its potency is satisfactory if it contains not less than 90 percent of the number of units that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e) (2) of that section, except use medium C in lieu of medium A, and medium F in lieu of medium E.

(3) *Moisture.* Proceed as directed in § 440.80a(b) (5) (i).

(4) *Pyrogens.* Proceed as directed in § 440.80a(b) (3), except use physiological salt solution as the diluent and inject 2 milliliters per kilogram of a solution containing 800 units per milliliter.

(5) *Toxicity.* Proceed as directed in § 440.80a(b) (4), except use physiological salt solution as the diluent and inject, in a 10-second interval, 1.0 milliliter of a solution containing 800 units per milliliter.

(6) *pH*—(i) *Dry mixture of the drug.* Proceed as directed in § 440.80a(b) (5) (ii), using the suspension resulting when the product is reconstituted as directed in the labeling.

(ii) *Aqueous suspension of the drug.* Proceed as directed in § 440.80a(b) (5) (ii), using the undiluted aqueous suspension.

§ 440.274 Procaine penicillin G injectable dosage forms.

§ 440.274a Procaine penicillin G in oil.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Procaine penicillin G in oil is a suspension of procaine penicillin G in a refined vegetable oil, with or without the addition of one or more suitable and harmless dispersing agents and with or without the addition of a hardening agent. Its potency is 300,000 units per milliliter. Its moisture content is not more than 1.4 percent. It is sterile. The procaine penicillin G used conforms to the requirements of § 440.-74a(a) (1). Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium. If the hardening agent is a hydrogenated and deodorized peanut oil, it is free from rancidity; it has an iodine value of not more than 10; its free fatty acid content as oleic acid is not more than one-tenth of 1 percent; and its melting point is 64±2° C.

(2) *Packaging.* The immediate container of procaine penicillin in oil shall be of colorless transparent glass (unless it is packaged to contain a single dose), so closed as to be a tight container as defined by the U.S.P., shall be sterile at the time of filling and closing, and shall be so sealed that its contents cannot be used without destroying such seal. The immediate container shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limitation therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good marketing, storage, and distribution practice shall be disregarded. The quantity of procaine penicillin in oil in each such container shall be not less than 1 milliliter and not more than 20 milliliters, unless it is packaged for repacking. Unless it is packaged for repacking, each such container shall be filled with a volume of procaine penicillin in oil in excess of that designated, which excess shall be sufficient to permit the withdrawal and the administration of the volume indicated, whether administered in single or multiple doses.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the outside wrapper or container and the immediate container, the statement "Expiration date _____", the blank being filled in, if it is packaged in plastic containers, with the date that is 12 months, or if its container is not plastic, with the date that is 36 months after the month during which the batch was certified, except that the blank may be filled in with the date that is 18 months, 24 months, or 36 months if the container is plastic or 48 months or 60 months if the container is not plastic, after the month during which the batch was certified, if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time such drug as prepared by him complies with the standards prescribed by paragraph (a) (1) of this section.

(ii) On the outside wrapper or container and the immediate container, the statement "Shake well," if it does not contain a hardening agent.

(4) *Requests for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch of procaine penicillin in oil shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless it was previously submitted) the date on which the latest assay of the procaine penicillin used in making such batch was completed, the number of units in each of such packages, the quantity of each ingredient used in making the batch, the date on which the latest assay of the drug comprising such batch was completed, and that each ingredient used in making such batch conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided by paragraph (a) (4) (iv) of this section, such person shall submit in connection with his request results of the tests and assays listed after each of the following made by him on an accurately representative sample of:

(*a*) The batch; potency, sterility (unless it is intended solely for udder instillations of cattle and is not required to be sterile), and moisture.

(*b*) The procaine penicillin G used in making the batch; potency, moisture, pH, crystallinity, penicillin K content (unless it is crystalline penicillin G), procaine penicillin G content, and, toxicity, sterility, and pyrogens.

(iii) Except as otherwise provided by paragraph (a) (4) (iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch:

(*1*) For all tests except sterility: One package for each 5,000 packages in the batch, but in no case less than five packages.

Such samples shall be collected by taking single packages at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(2) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation when each contains not less than 600,000 units or not less than 2.0 milliliters, or 40 immediate containers when each contains less than these amounts.

(*b*) The procaine penicillin used in making the batch:

(*1*) For all tests except sterility: 10 packages, each containing approximately equal portions of 300 milligrams.

(2) For sterility testing: 20 packages, each containing approximately 600 milligrams.

Each such portion shall be taken from a different part of such batch, and each shall be packaged in accordance with the requirements of § 440.74a(a) (2).

(*c*) In case of an initial request for certification, the vegetable oil and each dispersing and hardening agent or other ingredient used in making the batch: One package of each containing, respectively, approximately 250 grams and 5 grams.

(iv) No result referred to in paragraph (a) (4) (ii) (*b*) of this section, and no sample referred to in paragraph (a) (4) (iii) (*b*) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 440.80a

(b)(1), except § 440.80a(b)(1)(ix) and in lieu of the directions in § 440.80a(b)(1)(iv), prepare the sample by one of the following methods:

(i) *Bioassay*—(a) *Chloroform-alcohol solution.* By means of a 2-milliliter hypodermic syringe, introduce 1 milliliter of the well shaken sample into a 50-milliliter volumetric flask. Add 3 milliliters to 4 milliliters of chloroform, shake the flask thoroughly, and make to 50 milliliters with absolute alcohol. Mix thoroughly, withdraw a 1-milliliter aliquot and make the proper estimated dilutions in 1 percent phosphate buffer, pH 6.0.

(*b*) *Blending.* Introduce 1 milliliter of the well shaken sample by means of a 2.0-milliliter hypodermic syringe into a blending jar containing 98 milliliters of 1 percent phosphate buffer, pH 6.0, and 1 milliliter of polysorbate 80. Using a high-speed blender, blend the mixture for 1 minute and make the proper estimated dilutions in 1 percent phosphate buffer at pH 6.0.

(ii) *Iodometric chemical assay*—(*a*) *Chloroform-alcohol solution.* Introduce a representative quantity (usually 1 milliliter) of the well shaken sample into a flask and dissolve in sufficient chloroform-absolute ethanol (1+1) solvent to give a concentration of 2,000 units to 8,000 units per milliliter. An aliquot of this solution containing 2,000 units (estimated) is used as the solution for assay.

(*b*) *Extraction.* Accurately measure two representative portions of the sample (usually 1 milliliter) each equivalent to from 100,000 units to 300,000 units (estimated). Place one portion in a centrifuge tube containing 10 milliliters of chloroform and 10.0 milliliters of 1 percent phosphate buffer, pH 6.0. Shake the tube for 1 minute and centrifuge to obtain a substantially clear buffer layer. Dilute 5.0 milliliters of the buffer layer with sufficient 1 percent phosphate buffer, pH 6.0 to give a concentration of about 2,000 units per milliliter, and mix. Use 2.0 milliliters of this solution as the blank. Place the second portion of the sample in a centrifuge tube containing 10 milliliters of chloroform and 10.0 milliliters of 1*N* NaOH. Shake the tube for 1 minute and allow to stand for 15 minutes. Shake the tube again and centrifuge to obtain a substantially clear NaOH layer. Dilute 5.0 milliliters of the NaOH layer with sufficient 1*N* NaOH to give a concentration of about 2,000 units per milliliter. Use 2.0 milliliters of this solution as the inactivated solution. From the titration data calculate the amount of penicillin in the sample. If the label represents the potency of the procaine penicillin in oil as 100,000 units per milliliter, it is satisfactory if it is 85 percent or more of the potency so represented; if represented as 300,000 units per milliliter, it is satisfactory if it is 90 percent or more of the potency so represented.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except use medium B in lieu of medium A.

(3) *Moisture.* Proceed as directed in § 436.500(c).

§ 440.274b Procaine penicillin for aqueous injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Procaine penicillin for aqueous injection is a dry mixture of procaine penicillin and one or more suitable and harmless suspending or dispersing agents, with or without one or more suitable and harmless preservatives and buffer substances, or it is an aqueous suspension of procaine penicillin and one or more suitable and harmless suspending or dispersing agents, buffer substances, and preservatives, except that preservatives are not required if the immediate container is packaged to contain a single dose and is conspicuously so labeled. If it is an aqueous suspension of the drug, it may contain procaine hydrochloride in a concentration not exceeding 2.0 percent, one or more suitable and harmless stabilizing agents, and, if it is intended solely for veterinary use, it may contain cortisone or a suitable derivative of cortisone. It is so purified that:

(i) If it is an aqueous suspension of the drug, each container or each milliliter shall contain not less than 300,000 units;

(ii) It is sterile;

(iii) If it is the dry mixture of the drug, its moisture content is not more than 4.2 percent;

(iv) It is nonpyrogenic;

(v) It is nontoxic; and

(vi) Its pH in saturated aqueous solution is not less than 5.0 and not more than 7.5.

The procaine penicillin used conforms to the requirements of § 440.74a(a)(1). Each other substance, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* In all cases the immediate containers shall be tight containers as defined by the U.S.P., shall be sterile at the time of filling and closing, shall be so sealed that the contents cannot be used without destroying such seal and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded. In case it is packaged for dispensing, it shall be in immediate containers of colorless, transparent glass, closed by a substance through which a hypodermic needle may be introduced and withdrawn without removing the closure or destroying its effectiveness, unless it is the aqueous suspension of the drug and it is packaged to contain a single dose. If it is the dry mixture of the drug, each such container shall contain 300,000 units, 600,000 units, 900,000 units, 1,200,000 units, 1,500,000 units, or 3,000,000 units, unless it is intended solely for veterinary use and it is conspicuously so labeled. Each such container may be packaged in combination with a container of a suitable aqueous diluent. If it is the aqueous suspension of the drug, each such container shall contain not less than 1 milliliter (unless it is packaged to contain a single dose) and not more than 12 milliliters (unless it is intended solely for veterinary use and is conspicuously so labeled), and each shall be filled with a volume in excess of that designated, which excess shall be sufficient to permit the withdrawal and the administration of the volume indicated, whether administered in either single or multiple doses.

(3) *Labeling*—In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the outside wrapper or container and the immediate container, the statement "Expiration date _____", the blank being filled in, if it is a dry mixture of the drug, with the date that is 36 months, or if it is the aqueous suspension of the drug, with the date that is 12 months after the month during which the batch was certified, except that the blank may be filled in with the date that is 48 months or 60 months if it is the dry mixture of the drug, and 18 months, 24 months, 36 months, or 48 months if it is the aqueous suspension of the drug, after the month during which the batch was certified, if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time such drug as prepared by him complies with the standards prescribed by paragraph (a)(1) of this section.

(ii) On the immediate container, if it is a dry mixture of the drug, the conditions under which suspensions made from such drug should be stored, and the statement "Sterile suspension may be kept at room temperature for 1 week, or in a refrigerator for 3 weeks, without significant loss of potency" unless this information is contained in the circular or other labeling within or attached to the package.

(iii) On the outside wrapper or container, if it is the aqueous suspension of the drug, the statement "Store in refrigerator not above 15° C. (59° F.)" unless the person who requests certification has submitted to the Commissioner results of tests and assays showing that such drug as prepared by him complies with standards prescribed by paragraph (a)(1) of this section after having been stored at room temperature.

(iv) If it contains cortisone or a derivative of cortisone, after the name "procaine penicillin for aqueous injection," wherever it appears, the words "with _____", in juxtaposition with such name, the blank being filled in with the established name of such ingredient.

(4) *Requests for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch of procaine penicillin for

aqueous injection shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless it was previously submitted) the date on which the latest assay of the procaine penicillin used in making such batch was completed, the number of units in each of such packages, the quantity of each ingredient used in making the batch, the date on which the latest assay of the drug comprising such batch was completed, and a statement that each ingredient used in making the batch conforms to the requirements prescribed therefor, if any, by this section. If such batch, or any part thereof, is to be packaged with a solvent, such request shall also be accompanied by a statement that such solvent conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided by paragraph (a)(4)(v) of this section, such person shall submit in connection with his request results of the tests and assays listed after each of the following, made by him on an accurately representative sample of:

(*a*) The batch: potency, sterility, moisture, pyrogens, toxicity, pH.

(*b*) The procaine penicillin used in making the batch for potency, moisture, pH, crystallinity, penicillin K content (unless it is procaine penicillin G), and the penicillin G content if it is procaine penicillin G.

(iii) Except as otherwise provided by paragraph (a)(4)(v) of this section, if such batch is packaged for dispensing, such person shall submit in connection with his request, in the quantities hereinafter indicated accurately representative samples of the following:

(*a*) The batch:

(*1*) For all tests except sterility: One immediate container for each 5,000 immediate containers in such batch, but in no case less than 10 immediate containers.

Such samples shall be collected by taking single immediate containers at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation or 40 immediate containers if each contains less than 600 milligrams.

(*b*) The procaine penicillin used in making the batch: five packages, each containing approximately equal portions of not less than 500 milligrams, packaged in accordance with the requirements of § 440.74a(a)(2).

(*c*) In case of an initial request for certification, each other ingredient used in making the batch; one package of each containing approximately 5 grams.

(*d*) In case of an initial request for the certification of a batch of procaine penicillin for aqueous injection which is to be packaged in combination with an aqueous diluent which is not recognized by the U.S.P., or when any change is made in the composition of such diluent, five packages of the diluent included in the combination.

(iv) If such batch is packaged for repacking, such person shall submit with his request a sample consisting of the following:

(*a*) For all tests except sterility: 10 packages each containing approximately 300 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 600 milligrams.

Each such portion shall be taken from a different part of such batch, and each shall be packaged in accordance with the requirements of paragraph (a)(2) of this section.

(v) No result referred to in paragraph (a)(4)(ii)(*b*) of this section, and no sample referred to in paragraph (a)(4)(iii)(*b*) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay*—(1) *Potency.* Using as the sample for assay a representative aliquot of the suspension equivalent to one dose (or, if it is a dry mixture of the drug, a representative aliquot of the drug equivalent to one dose after it has been reconstituted as directed in the labeling), proceed as directed in § 440.80a(b)(1), using approximately 50 to 100 milliliters of redistilled methyl alcohol, dimethyl formamide, or formamide to dissolve the sample or by the iodometric method as described in § 440.80a(b)(5)(iv)(*a*), except dissolve the sample in about 5.0 milliliters of redistilled methyl alcohol, dimethyl formamide, or formamide prior to diluting with 1 percent phosphate buffer, pH 6.0. Its potency is satisfactory if it contains not less than 90 percent of the number of units that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except add sufficient penicillinase to diluting fluid A and swirl the flask to completely solubilize the procaine penicillin before filtration. If the product contains lecithin, use diluting fluid D in lieu of diluting fluid A. If the product contains sodium carboxymethylcellulose, add sufficient sterile carboxymethylcellulase to diluting fluid A or D to completely solubilize the sodium carboxymethylcellulose before filtration. If the preparation contains homogenizers or suspending agents that prevent solubilization, proceed as directed in paragraph (e)(2) of that section, except use medium B in lieu of medium A.

(3) *Moisture.* Proceed as directed in § 440.74a(b)(5).

(4) *Pyrogens.* Proceed as directed in § 440.80a(b)(3), except use physiological salt solution as the diluent.

(5) *Toxicity.* Proceed as directed in § 440.80a(b)(4), except use physiological salt solution as the diluent, and inject 0.25 milliliter of a solution containing 4,000 units per milliliter.

(6) *pH*—(i) *Dry mixture of the drug.* Proceed as directed in § 440.80a(b)(5)(ii) using the solution or suspension resulting when the amount of diluent recommended in the labeling is added.

(ii) *Aqueous suspension of the drug.* Proceed as directed in § 440.80a(b)(5)(ii), using the undiluted aqueous suspension.

§ 440.280 Penicillin injectable dosage forms.

§ 440.280a Sterile sodium penicillin, calcium penicillin, crystalline penicillin (crystalline penicillin sodium, crystalline penicillin potassium, crystalline penicillin G sodium, crystalline penicillin G potassium, crystalline penicillin O sodium, crystalline penicillin O potassium).

The requirements for certification and the tests and methods of assay for sterile sodium penicillin, calcium penicillin, crystalline penicillin (crystalline penicillin sodium, crystalline penicillin potassium, crystalline penicillin G sodium, crystalline penicillin G potassium, crystalline penicillin O sodium, crystalline penicillin O potassium) packaged for dispensing are described in § 440.80a.

§ 440.280b Potassium penicillin G for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Potassium penicillin G for injection is a dry mixture of potassium penicillin G and the buffer sodium citrate in a quantity not less than 4 percent and not more than 5 percent by weight of its total solids. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of units of penicillin G that it is represented to contain. It is sterile. It is nonpyrogenic. It passes the safety test. Its loss on drying is not more than 1.5 percent. When reconstituted as directed in the labeling, its pH is not less than 7 and not more than 8.5. The potassium penicillin G used conforms to the standards prescribed by § 440.80a(a)(1) of this chapter. The sodium citrate conforms to the standards prescribed therefor by the U.S.P.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The potassium penicillin G used in making the batch for potency, loss on drying, pH, penicillin G content, and crystallinity.

(*b*) The batch for potency, sterility, pyrogens, safety, loss on drying, and pH.

(ii) Samples required:

(*a*) The potassium penicillin G used in making the batch: 10 packages, each containing approximately 60 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Reconstitute as directed in the labeling. Dilute the resultant solution with 1 percent potassium phosphate buffer, pH 6.0

(solution 1), to give a stock solution of convenient concentration.

(ii) *Assay procedures.* Assay for potency by any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 1 unit of penicillin-G per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(*c*) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 2,000 units of penicillin-G per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this chapter.

(5) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using the solution obtained when the drug is reconstituted as directed in the labeling.

§ 440.280c Crystalline penicillin and bacitracin.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Crystalline penicillin and bacitracin is composed of crystalline sodium penicillin or potassium penicillin and bacitracin with or without suitable and harmless buffer substances and dispersing agents. It is so purified and dried that:

(i) It is sterile;

(ii) It is nontoxic;

(iii) It is nonpryogenic;

(iv) Its moisture content is not more than 5.0 percent;

(v) The pH of an aqueous solution prepared as directed in its labeling is not less than 5.0 and not more than 7.5.

The crystalline penicillin used conforms to the standard prescribed by § 440.80a (a)(1). The bacitracin used conforms to the requirements prescribed by § 448.10a (a)(1) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* In all cases the immediate containers shall be tight containers as defined by the U.S.P., shall be sterile at the time of filling and closing, shall be so sealed that the contents cannot be used without destroying the seal, and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practices shall be disregarded. In case it is packaged for dispensing, it shall be in immediate containers of colorless transparent glass closed by a substance through which a hypodermic needle may be introduced and withdrawn without removing the closure or destroying its effectiveness; each such container shall contain 1,500,000 units of crystalline penicillin and 60,000 units of bacitracin and each may be packaged in combination with a container of the solvent, water for injection U. S. P., physiological salt solution U. S. P., or with an aqueous solution of a suitable local anesthetic.

(3) *Labeling—*(i) *It is packaged for dispensing.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its label or labeling, as hereinafter indicated, the following:

(*a*) On the outside wrapper or container and the immediate container, the statement "Expiration date _____", the blank being filled in with the date that is 18 months after the month during which the batch was certified.

(*b*) On the circular or other labeling within or attached to the package, the conditions under which such solutions should be stored, including a reference to their instability when stored under other conditions, and the statement "Sterile solution may be kept in refrigerator for 3 days without significant loss of potency".

(ii) *It is packaged solely for manufacturing use and/or repacking.* Each package shall bear on its outside wrapper or container and the immediate container, the following:

(*a*) The number of units of crystalline penicillin per milligram and the number of grams in the immediate container.

(*b*) The number of units of bacitracin per milligram and the number of grams in the immediate container.

(*c*) The statement "Caution: Federal law prohibits dispensing without prescription".

(*d*) The statement "For manufacturing use", "For repacking", or "For manufacturing use or repacking".

(*e*) The information required by paragraph (3)(i) of this section.

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch of crystalline penicillin and bacitracin shall submit with his request a statement showing the batch mark, the number of packages in such batch, the number of units of penicillin and the number of units of bacitracin in each package, the batch marks, and (unless they were previously submitted) the dates on which the latest assays of the penicillin and bacitracin used in making such batch were completed, the date on which the latest assay of the drug comprising such batch was completed, the quantity of each ingredient used in making the batch and a statement that each such ingredient conforms to the requirements prescribed therefor by this section. If such batch, or any part thereof, is to be packaged with a solvent, such request shall also be accompanied by a statement that such solvent conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided by paragraph (a)(4)(v) of this section, such person shall submit in connection with his request results of the tests and assays listed after each of the following made by him on an accurately representative sample of:

(*a*) The batch; potency, sterility, toxicity, pyrogens, moisture, pH.

(*b*) The crystalline penicillin used in making the batch; potency, crystallinity, heat stability, penicillin K content (unless it is crystalline penicillin G), and the penicillin G content if it is crystalline penicillin G.

(*c*) The bacitracin used in making the batch; potency, toxicity, ash content, and heavy metals content.

(iii) Except as otherwise provided by paragraph (a)(4)(v) of this section, if such batch is packaged for dispensing, such person shall submit in connection with his request in the quantities hereinafter indicated accurately representative samples of the following:

(*a*) The batch:

(*1*) For all tests except sterility: One immediate container for each 5,000 immediate containers in such batch, but in no case less than 10 immediate containers.

Such samples shall be collected by taking single immediate containers at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation, or 40 immediate containers if each contains less than 600 milligrams.

(*b*) The crystalline penicillin used in making the batch; 3 packages, each containing approximately equal portions of not less than 250 milligrams, packaged in accordance with the requirements of § 440.80a(a)(2).

(*c*) The bacitracin used in making the batch; 4 packages, each containing approximately 0.5 gram packaged in accordance with the requirements of § 448.10a(a)(2) of this chapter.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch; 1 package of each containing approximately 5 grams.

(iv) If such batch is packaged for repacking, such person shall submit with his request a sample consisting of the following:

(*a*) For all tests except sterility: 10 approximately equal portions of at least 2 grams each.

(*b*) For sterility testing: 20 packages, each containing approximately 600 milligrams.

Each such portion shall be taken from different parts of such batch, and each shall be packaged in a separate con-

tainer and in accordance with the requirements of paragraph (a)(2) of this section.

(v) No result referred to in paragraph (a)(4)(ii)(*b*) and (*c*) of this section, and no sample referred to in paragraph (a)(4)(iii)(*b*) and (*c*) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Content of penicillin.* Proceed as directed in § 440.80a(b)(1). Its content of penicillin is satisfactory if it contains not less than 90 percent of the number of units it is represented to contain.

(ii) *Content of bacitracin.* Proceed as directed in § 448.10a(a)(1) of this chapter, except that sufficient penicillinase is added to the sample under test to completely inactivate the penicillin present. Its content of bacitracin is satisfactory if it contains not less than 85 percent and not more than 115 percent of the number of units it is represented to contain.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Toxicity.* Proceed as directed in § 440.80a(b)(4) except use physiological salt solution as the diluent.

(4) *Pyrogens.* Proceed as directed in § 440.80a(b)(3) using as a test dose 1.0 milliliter per kilogram of a solution containing 300 units of bacitracin per milliliter. Use physiological salt solution as the diluent.

(5) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i).

(6) *pH.* Proceed as directed in § 440.80a(b)(5)(ii), using a solution prepared as directed in the labeling for the drug.

§ 440.280d Crystalline penicillin and epinephrine in oil.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Crystalline penicillin and epinephrine in oil is a suspension of crystalline penicillin and epinephrine in a menstruum of refined peanut oil or sesame oil. Each milliliter has a potency of 300,000 units and contains 0.3 milligram of epinephrine. Its moisture content is not more than 0.2 percent. It is sterile. The penicillin used conforms to the requirements of § 440.80a(a)(1) for crystalline penicillin. The epinephrine, peanut oil, and sesame oil used conform to the standards prescribed therefor by the U.S.P.

(2) *Packaging.* The immediate container of crystalline penicillin and epinephrine in oil shall be of colorless transparent glass so closed as to be a tight container as defined by the U.S.P., shall be sterile at the time of filling and closing, shall be so sealed that its contents cannot be used without destroying such seal, and shall be of such composition as will not cause any change in the strength quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded. The quantity of crystalline penicillin and epinephrine in oil in each such container shall be not less than one milliliter and not more than 20 milliliters unless it is packaged for repacking. Unless it is packaged for repacking each container shall be filled with a volume of crystalline penicillin and epinephrine in oil in excess of that designated, which excess shall be sufficient to permit the withdrawal and the administration of the volume indicated whether administered in either single or multiple doses.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on the outside wrapper or container and the immediate container the statement "Expiration date _____", the blank being filed in with the date that is 18 months after the month during which the batch was certified.

(4) *Requests for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch of crystalline penicillin and epinephrine in oil shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless it was previously submitted) the date on which the latest assay of the penicillin used in making such batch was completed, the number of units in each of such packages, the quantity of each ingredient used in making the batch, the date on which the latest assay of the drug comprising such batch was completed, and that the epinephrine, peanut oil or sesame oil used in making such batch conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request results of the tests and assay listed after each of the following, made by him on an accurately representative sample of:

(*a*) The batch; potency, sterility, moisture and epinephrine content.

(*b*) The penicillin used in making the batch; potency, sterility, toxicity, pyrogens, moisture, pH, penicillin K content (unless it is crystalline penicillin G), crystallinity, heat stability and the penicillin G content, if it is crystalline penicillin G.

(iii) Except as otherwise provided by paragarph (a)(4)(iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch:

(*1*) For all tests except sterility: One package for each 500 packages in the batch, but in no case less than five packages or more than 15 packages.

Such samples shall be collected by taking single packages at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(*2*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation when each container contains not less than 600,000 units or not less than 2.0 milliliters, or 40 immediate containers when each contains less than these amounts.

(*b*) The penicillin used in making the batch:

(*1*) For all tests except sterility: 10 packages, each containing approximately equal portions of not less than 60 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 600 milligrams.

Each such portion shall be taken from a different part of such batch and each shall be packaged in accordance with the requirements of § 440.80a(a)(2).

(c) In case of an initial request for certification, the epinephrine and the peanut oil or sesame oil used in making the batch; one package of each containing approximately 2 grams and 250 grams, respectively.

(iv) No result referred to in paragraph (a)(4)(ii)(*b*) of this section, and no sample referred to in paragraph (a)(4)(iii)(*b*) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.500(a) of this chapter except the provisions for warming the sample. When examined by the method described in this section the potency of crystalline penicillin and epinephrine in oil is satisfactory if it is 90 percent or more of the potency represented.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except use medium B in lieu of medium A.

(3) *Moisture.* Proceed as directed in § 436.500(c) of this chapter.

(4) *Epinephrine content*—(i) *Standard curve.* Prepare a stock standard containing 20 milligrams of U. S. P. epinephrine reference standard in 100 milliliters of phosphate buffer at pH 4.0 This buffer is prepared by adjusting 1 percent phosphate buffer pH 6.0 with 1:100 phosphoric acid (ortho 85 percent) to pH 4.0. The stock standard is stored in the refrigerator and may be used as long as it remains colorless. Transfer 1.0, 2.0, 3.0, 4.0, and 5.0 milliliters of the standard to each of five 100-milliliter volumetric flasks using transfer pipettes. Add 4.0, 3.0, 2.0, and 1.0 milliliters of the phosphate buffer pH 4.0 to the first four flasks respectively to give each a total volume of 5 milliliters. Add 1.0 milliliter of 0.1 *N* iodine to each flask, shake for one minute, add 2.0 milliliters of 0.1 *N* sodium thiosulfate and mix the solution. Make each flask to a volume of 100 milliliters with distilled water. Read the percent light transmission of the colored solutions using a 2.0 centimeter cell and a 490 mμ filter in a suitable photoelectric colorimeter. The instrument is balanced so that the 0.2 mg. 100 ml. concentration reads 90 percent light transmission. Prepare a standard curve on semilog paper,

plotting the percent light transmission on the logarithmic ordinate scale and the concentration on the abscissa.

(ii) *Procedure.* Transfer 1.0 milliliter of the sample with the aid of a hypodermic syringe and needle to a screw-cap test tube (about 30 milliliters capacity), add 5.0 milliliters of U. S. P. chloroform and 10.0 milliliters of 1 percent phosphate buffer pH 6.0 from a transfer pipette. Shake thoroughly for one minute, centrifuge to separate the layers and carefully withdraw a 5.0-milliliter aliquot of the buffer layer using a transfer pipette. Transfer the aliquot to a 50-milliliter volumetric flask, add the calculated amount of 1:100 phosphoric acid to bring the pH to 4.0 (amount to be added previously determined by titration of the 1 percent phosphate buffer pH 6.0 with 1:100 phosphoric acid using a pH meter). Add 1.0 milliliter of 0.1*N* iodine, shake for one minute, add 2.0 milliliters of 0.1 *N* sodium thiosulfate and mix the solution. Make to a volume of 50 milliliters with distilled water. Set the colorimeter at 90 percent light transmission for the 0.2 milligram/100 milliliters standard as directed above and obtain the percent light transmission of the sample. The concentration obtained directly from the standard curve corresponding to the percent light transmission of the sample × 1.05 equals the concentration of the epinephrine per milliliter of the sample. Crystalline penicillin and epinephrine in oil is satisfactory if it contains not less than 0.24 or more than 0.36 milligram epinephrine per milliliter.

§ 440.281 Buffered crystalline penicillin.

The requirements for certification and the tests and methods of assay for buffered crystalline penicillin packaged for dispensing are described in § 440.81.

Subpart D–E—[Reserved]

Subpart F—Dermatologic Dosage Forms

§ 440.563 Ephedrine penicillin tablets.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Ephedrine penicillin tablets are tablets composed of ephedrine penicillin and one or more buffer substances, with or without one or more suitable and harmless diluents, binders, and lubricants. The potency of each tablet is not less than 30,000 units. Its moisture content is not more than 1.5 percent. When a tablet is dissolved as directed the potency of such solution is not less than 4,000 units per milliliter after it has been kept for one day at a temperature of 15° C. (59° F). Such solution is isotonic, and has a pH of 6.0, ±0.2. The ephedrine penicillin used conforms to the requirements of § 440.63a(a)(1) for ephedrine penicillin except paragraph (a)(1)(ii) and (iv) of § 440.63a. Each buffer substance and preservative used, if their names are recognized in the U.S.P. or N.F., conform to the standards prescribed therefor by such official compendium.

(2) *Packaging.* Unless each tablet is enclosed in foil or plastic film and such enclosure is a tight container as defined by the U.S.P., except the provision that it shall be capable of tight reclosure, the immediate container shall be a tight container as so defined. The immediate container may also contain a desiccant separated from the tablets by a plug of cotton or other like material. The composition of the immediate container, or of the foil or film enclosure, shall be such as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106 (b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on the outside wrapper or container and the immediate container, as hereinafter indicated, the following:

(i) The statement "Expiration date _____", the blank being filled in with the date that is 24 months after the month during which the batch was certified.

(ii) A statement giving the method of dissolving the tablets, and a statement that distilled water U.S.P. should be used.

(iii) The conditions under which the solution should be stored, including a reference to its instability when stored under other conditions, and a statement "Prepare a fresh solution each 24 hours".

(iv) The statement "*Warning*—Not for injection or oral use".

(4) *Request for certification, samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch of ephedrine penicillin tablets shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless it was previously submitted) the date on which the latest assay of the ephedrine penicillin used in making such batch was completed, the number of units in each tablet, the quantity of each ingredient used in making the batch, the date on which the latest assay of the drug comprising the batch was completed, and a statement that each ingredient used in making the batch conforms to the requirements prescribed therefor, if any by this section.

(ii) Except as otherwise provided in paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request results of the tests and as says listed after each of the following, made by him on an accurately representative sample of:

(*a*) The batch; average potency per tablet and average moisture.

(*b*) The ephedrine penicillin used in making the batch; potency, toxicity, moisture, pH, penicillin K content (unless it is ephedrine penicillin G), crystallinity and the penicillin G content if it is ephedrine penicillin G.

(iii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch; one table for each 5,000 tablets in the batch, but in no case less than 30 tablets, collected by taking single tablets at such intervals throughout the entire time of tableting that the quantities tableted during the intervals are approximately equal.

(*b*) The ephedrine penicillin used in making the batch; six packages, each containing approximately equal portions of not less than 300 milligrams, packaged in accordance with the requirements of § 440.63a(a)(2).

(iii) In case of an initial request for certification, each buffer substance, diluent, binder, and lubricant used in making the batch; one packaging of each, each containing approximately 5 grams.

(iv) No result referred to in paragraph (a)(4)(ii) (*b*) of this section, and no sample referred to in paragraph (a)(4)(iii) (*b*) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 440.180a(b)(1) or by the iodometric method as described in § 440.80a(b)(5)(iv)(*a*), using 6 tablets dissolved in sufficient 1 percent phosphate buffer pH 6.0 to give a concentration of 2,000 units per milliliter.

(2) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i).

PART 442—CEPHA ANTIBIOTICS

Subpart A—Bulk Drugs

Sec.
442.11a Sterile cefazolin sodium.
442.21 Cephaloglycin dihydrate.
442.23a Sterile cephaloridine.
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Subpart B—Oral Dosage Forms

442.121 Cephaloglycin dihydrate oral dosage forms.
442.121a Cephaloglycin dihydrate capsules.
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Subpart C—Injectable Dosage Forms

442.211 Sterile cefazolin sodium.
442.223 Sterile cephaloridine.
442.225 Sterile sodium cephalothin.
442.229 Sterile cephapirin sodium.

AUTHORITY: Sec. 507, 59 Stat. 463, as amended (21 U.S.C. 357).

Subpart A—Bulk Drugs

§ 442.11a Sterile cefazolin sodium.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Sterile cefazolin sodium is the sodium salt of 3-[[(5-methyl-1,3,4-thiadiazol-2-yl)-thio]methyl]-7-[2-(1H-tetrazol-1-yl) acetamido] - 3 - cephem-4-carboxylic acid. It is so purified and dried that:

(i) Its potency is not less than 850 micrograms and not more than 1050 mi-

crograms of cefazolin per milligram calculated on an anhydrous basis. If it is packaged for dispensing, its cefazolin content is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of cefazolin that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It passes the safety test.

(v) Its moisture content is not more than 6 percent.

(vi) Its pH is an aqueous solution containing 100 milligrams of cefazolin per milliliter is not less than 4.0 and not more than 6.0.

(vii) The specific rotation in a 0.1*M* sodium bicarbonate solution containing 50 milligrams of cefazolin per milliliter at 25° C. is −17°±7° calculated on an anhydrous basis.

(viii) It gives a positive identity test for cefazolin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, safety, moisture, pH, specific rotation and identity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: 9 packages, each containing approximately 500 milligrams, and 1 package containing approximately 5 grams.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 12 immediate containers, except if each contains less than 500 milligrams, a minimum of 16 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Dissolve an accurately weighed sample in sufficient 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration; also if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents. Dilute with sufficent solution 1 to give a stock solution of convenient concentration.

(ii) *Assay procedure.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 micrograms of cefazolin per milliliter (estimated).

(*b*) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter, preparing the working standard solution as follows: Dissolve an accurately weighed portion of approximately 30 milligrams of cefazolin working standard in 3 milliliters of 10 percent potassium phosphate buffer, pH 6.0 (solution 6), and further dilute with solution 1 to the final concentration.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e) (1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 50 milligrams of cefazolin per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this chapter.

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams of cefazolin per milliliter.

(7) *Specific rotation.* Proceed as directed in § 436.210 of this chapter, using a solution containing 50 milligrams of cefazolin per milliliter in 0.1*M* sodium bicarbonate and a polarimeter tube 1.0 decimeter in length. Calculate the specific rotation on an anhydrous basis.

(8) *Identity.* Using a 0.002 percent solution of the sample in 0.1*M* sodium bicarbonate solution and a suitable spectrophotometer, record the ultraviolet spectrum from 220 to 340 nanometers. The spectrum compares qualitatively to that of the cefazolin working standard similarly tested.

§ 442.21 Cephaloglycin dihydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cephaloglycin dihydrate is the dihydrate form of 7-(D-α-aminophenylacetamido) cephalosporanic acid. It is a white to off-white powder. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms of cephaloglycin per milligram on an anhydrous basis.

(ii) It passes the safety test.

(iii) Its moisture is not less than 8.2 and not more than 12 percent.

(iv) Its pH in an aqueous suspension containing 50 milligrams per milliliter is not less than 3.0 and not more than 5.5.

(v) Its cephaloglycin content is not less than 95 and not more than 104 percent on an anhydrous basis.

(vi) It gives a positive identity test for cephaloglycin dihydrate.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, moisture, pH, cephaloglycin content, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient sterile distilled water to give a stock solution of 100 micrograms of cephaloglycin per milliliter (estimated). Further dilute an aliquot of the stock solution with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 10 micrograms of cephaloglycin per milliliter (estimated).

(2) *Safety.* Proceed as directed in § 436.33 of this chapter, except observe the mice for 7 days.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous suspension containing 50 milligrams per milliliter.

(5) *Cephaloglycin content.* Proceed as directed in § 436.213 of this chapter, using the titration procedure described in paragraph (e) (2) of that section. Calculate the cephaloglycin content as follows:

$$\text{Percent cephaloglycin content} = \frac{(A-B)\ (\text{normality of perchloric acid reagent})\ (405.4)\ (100)\ (100)}{(\text{Weight of sample in milligrams})\ (100-m)}$$

where:

A=Milliliters of perchloric acid reagent used in titrating the sample;

B=Milliliters of perchloric acid reagent used in titrating the blank;

m=Percent moisture content of the sample.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using the 0.5-percent potassium bromide disc prepared as described in paragraph (b) (1) of that section.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

§ 442.23a Sterile cephaloridine.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cephaloridine is 7-[α-(2-thienyl)-acetamido]-3-(1-pyridylmethyl)-3-cephem-4-carboxylic acid betaine. It is a white to off-white powder. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms of cephaloridine per milligram. If it is packaged for dispensing, its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of cephaloridine that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It passes the safety test.

(v) Its loss on drying is not more than 2.5 percent.

(vi) Its pH in an aqueous solution is not less than 3.5 and not more than 6.

(vii) The specific rotation in an aqueous solution containing 10 milligrams of cephaloridine per milliliter at 25° C. is +48°±4°.

(viii) It is crystalline.

(ix) The ultraviolet absorption spectrum between the wavelengths of 220 and 310 nanometers compares qualitatively to that of the cephaloridine working stand-

ard. The ratio of the absorbance of the maximum at the wavelength of 240 nanometers to that of the shoulder at 255 nanometers is not less than 1.05 and not more than 1.17.

(2) *Labeling.* It shall be labeled in accordance with the requirements prescribed by § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays of the batch for potency, sterility, pyrogens, safety, loss on drying, pH, specific rotation, crystallinity, and identity.

(ii) Samples of the batch:

(*a*) If the batch is packaged for repacking or for use as an ingredient in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing at least 500 milligrams.

(*2*) For sterility testing: 20 packages, each containing equal portions of approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 13 immediate containers of the batch.

(*2*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Dissolve an accurately weighted sample in sufficient 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), for the microbiological agar diffusion assay, distilled water for the iodometric assay or hydroxylamine colorimetric assay, to give a stock solution of convenient concentration; also if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with either solution 1 or distilled water as specified above to give a stock solution of convenient concentration.

(ii) *Assay procedures.* Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 microgram of cephaloridine per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter. If it is packaged for dispensing, dilute an aliquot of the stock solution with distilled water to the prescribed concentration.

NOTE: The 10 milliliters of 0.01*N* iodine must be added within 20 seconds after the addition of the 2.0 milliliters of 1.2*N* HCl, and the assay should be completed within 1 hour after the sample and standard are first put into solution. The working standard should be dried as described in § 436.200(a) of this chapter.

(*c*) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 50 milligrams of cephaloridine per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this chapter.

(5) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 250 milligrams of cephaloridine per milliliter. If it is packaged for dispensing, however, use the solution obtained after reconstituting the drug as directed in the labeling.

(7) *Specific rotation.* Dilute an accurately weighed sample with sufficient distilled water to give a concentration of approximately 10 milligrams of cephaloridine per milliliter. Proceed as directed in § 436.210 of this chapter using a 2.0-decimeter polarimeter tube.

(8) *Crystallinity.* Proceed as directed in § 436.203 (a) of this chapter.

(9) *Identity.* Using a 0.0025-percent solution of the sample in water and a suitable spectrophotometer, record the ultraviolet absorption spectrum from 220 to 310 nanometers. The spectrum compares qualitatively to that of the cephaloridine working standard similarly tested.

§ 442.25a Sterile sodium cephalothin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium cephalothin is the sodium salt of the compound formed by reaction of thiophene-2-acetic acid with 7-amino-cephalosporanic acid. The 7-amino-cephalosporanic acid is obtained from a kind of cephalosporin. It is so purified and dried that:

(i) Its potency is not less than 850 micrograms of cephalothin per milligram on an anhydrous basis. If it is packaged for dispensing, its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of cephalothin that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It passes the safety test.

(v) Its loss on drying is not more than 1.5 percent.

(vi) Its pH in an aqueous solution is not less than 4.5 and not more than 7.0.

(vii) The specific rotation in an aqueous solution containing 50 milligrams of cephalothin per milliliter at 25° C. is $+129° \pm 5°$.

(viii) It gives a positive identity test.

(ix) It is crystalline.

(2) *Packaging.* In addition to the requirements of § 432.2 of this chapter, if it is packaged for dispensing and is intended for both intravenous and intramuscular use, each vial shall contain the equivalent of 1 gram of cephalothin; except that if it is packaged for dispensing and is intended solely for intravenous use, each vial shall contain the equivalent of 4 grams of cephalothin.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 432.5 of this chapter, if it is packaged for dispensing, each package shall bear on its label and labeling, the following statement: "After reconstitution, store in a refrigerator and use within 48 hours. If kept at room temperature, use within 6 hours."

(4) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays of the batch for potency, sterility, pyrogens, safety, loss on drying, pH, specific rotation, crystallinity, and identity.

(ii) Samples of the batch:

(*a*) If the batch is packaged for repacking or for use as an ingredient in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing equal portions of approximately 500 milligrams.

(*2*) For sterility testing: 20 packages, each containing equal portions of approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 10 immediate containers of the batch.

(*2*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Dissolve an accurately weighed sample in sufficient 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), for the microbiological agar diffusion assay, distilled water for the iodometric assay or hydroxylamine colorimetric assay, to give a stock solution of convenient concentration; also if it is packaged for dispensing, reconstitute as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with either solution 1 or distilled water as specified above to give a stock solution of convenient concentration.

(ii) *Assay procedures.* Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 microgram of cephalothin per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter. If it is packaged for dispensing, dilute an aliquot of the stock solution with distilled water to the prescribed concentration.

NOTE: The 10 milliliters of 0.01*N* iodine must be added within 20 seconds after the addition of the 2.0 milliliters of 1.2*N* HCl, and the assay should be completed within 1 hour after the sample and standard are first put into solution. The working standard should be dried as described in § 436.200(a) of this chapter.

(c) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 50 milligrams of cephalothin per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this chapter.

(5) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 250 milligrams per milliliter; however, if it is packaged for dispensing, use the solution obtained after reconstituting the drug as directed in the labeling.

(7) *Specific rotation.* Dilute an accurately weighed sample with sufficient distilled water to give a concentration of approximately 50 milligrams per milliliter. Proceed as directed in § 436.210 of this chapter, using a 1.0-decimeter polarimeter tube and calculate the specific rotation on an anhydrous basis.

(8) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(9) *Identity.* Using a 0.0025-percent solution of the sample in water and a suitable spectrophotometer, record the ultraviolet absorption spectrum from 220 to 310 nanometers. The spectrum compares qualitatively to that of the cephalothin working standard similarly tested.

§ 442.27 Cephalexin monohydrate.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Cephalexin monohydrate is the monohydrate form of 7-(D-2-amino-2-phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms of cephalexin per milligram on an anhydrous basis.

(ii) It passes the safety test.

(iii) Its moisture content is not less than 4.0 nor more than 8.0 percent.

(iv) Its pH in an aqueous solution containing 50 milligrams per milliliter is not less than 3.0 nor more than 5.5.

(v) When calculated on an anhydrous basis, its absorptivity at 262 nanometers is not less than 95 percent and not more than 104 percent of that of the cephalexin standard similarly treated and corrected for potency.

(vi) It gives a positive identity test.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, moisture, pH, absorptivity, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay—*(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution containing 1.0 milligram per milliliter (estimated). Further dilute an aliquote of the stock solution with solution 1 to the reference concentration of 20 micrograms of cephaxelin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter.

NOTE: The 10 milliliters of 0.01*N* iodine must be added within 20 seconds after the addition of the 2.0 milliliters of 1.2*N* hydrochloric acid, and the assay should be completed within 1 hour after the sample and standard are first put into solution.

(2) *Safety.* Proceed as directed in § 436.33 of this chapter, except observe the mice for 7 days.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous suspension containing 50 milligrams per milliliter.

(5) *Absorptivity.* Determine the absorbance of the sample and standard solutions in the following manner: Dissolve accurately weighed portions of approximately 50 milligrams each of the sample and standard in 250 milliliters of distilled water. Transfer a 10-milliliter aliquot to a 100-milliliter volumetric flask and dilute to volume with distilled water. Using a suitable spectrophotometer and distilled water as the blank, determine the absorbance of each solution at 262 nanometers. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{milligrams standard} \times \text{potency of standard in micrograms per milligram} \times 10}{\text{Absorbance of standard} \times \text{milligrams sample} \times (100 - m)}$$

where m = percent moisture in the sample.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using the 0.5 percent potassium bromide disc prepared as described in paragraph (b)(1) of that section.

(7) *Crystallinity.* Proceed as directed in § 436.203 of this chapter.

§ 442.29a Sterile cephapirin sodium.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Sterile cephapirin sodium is the sodium salt of 7-[α(4-pyridylthio)-acetamido]-cephalosporanic acid. It is a white to off-white powder. It is so purified and dried that:

(i) Its potency is not less than 855 micrograms and not more than 1,000 micrograms of cephapirin per milligram on an "as is" basis. If it is packaged for dispensing, its content is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of cephapirin that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It passes the safety test.

(v) Its moisture content is not more than 2.0 percent.

(vi) Its pH in an aqueous solution containing 10 milligrams of cephapirin per milliliter is not less than 6.5 and not more than 8.5.

(vii) Its cephapirin content is not less than 92 percent and not more than 105 percent on an anhydrous basis.

(viii) It gives a positive identity test for sodium cephapirin.

(ix) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, safety, moisture, pH, cephapirin content, identity, and crystallinity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: 9 packages, each containing approximately 500 milligrams, and 1 package containing approximately 5 grams.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 14 immediate containers, except if each contains less than 1 gram, a minimum of 19 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay—*(1) *Potency.* Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration; also, if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with solution 1 to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 micro-

gram of cephapirin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter. In addition if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with distilled water to the prescribed concentration.

(iii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter. In addition, if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with distilled water to the prescribed concentration.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 100 milligrams of cephapirin per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this chapter.

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(7) *Cephapirin content.* Transfer an accurately weighed 400- to 500-milligram sample to a 250-milliliter Erlenmeyer flask, add 50 milliliters of glacial acetic acid, and, while stirring, warm in a 70° C. water bath until the sample is completely dissolved. Add three drops of a 0.5 percent solution of crystal violet in glacial acetic acid as indicator, and titrate with 0.1*N* perchloric acid in glacial acetic acid (previously standardized against diphenylguanidine) to the first clear green end point. Each milliliter of 0.1*N* perchloric acid is equivalent to 22.275 milligrams of cephapirin. Calculate the cephapirin content on the anhydrous basis.

(8) *Identity.* Proceed as directed in § 436.211 of this chapter, using a 1.0 percent potassium bromide disc prepared as directed in paragraph (b)(1) of that section.

(9) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

Subpart B—Oral Dosage Forms

§ 442.121 Cephaloglycin dihydrate oral dosage forms.

§ 442.121a Cephaloglycin dihydrate capsules.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Cephaloglycin dihydrate capsules are composed of cephaloglycin dihydrate and one or more suitable lubricants and diluents enclosed in a gelatin capsule. Each capsule contains cephaloglycin dihydrate equivalent to 250 milligrams of cephaloglycin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cephaloglycin that it is represented to contain. Its moisture content is not more than 9 percent. The cephaloglycin used conforms to the standards prescribed by § 442.21(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The cephaloglycin dihydrate used in making the batch for potency, safety, moisture, pH, cephaloglycin content, identity, and crystallinity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The cephaloglycin dihydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar with sufficient 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 4 to the reference concentration of 10 micrograms of cephaloglycin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 442.121b Cephaloglycin dihydrate for oral suspension.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Cephaloglycin dihydrate for oral suspension is cephaloglycin dihydrate with one or more suitable diluents, buffer substances, colorings, and flavorings. When reconstituted as directed in the labeling, each milliliter contains cephaloglycin dihydrate equivalent to 50 milligrams of cephaloglycin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cephaloglycin that it is represented to contain. Its moisture content is not more than 2 percent. When reconstituted as directed in the labeling, its pH is not less than 3.0 and not more than 5.0. It passes the identity test for the presence of the cephaloglycin moiety. The cephaloglycin dihydrate used conforms to the standards prescribed by § 442.21(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The cephaloglycin dihydrate used in making the batch for potency, safety, moisture, pH, cephaloglycin content, identity, and crystallinity.

(*b*) The batch for potency, moisture, pH, and identity.

(ii) Samples required:

(*a*) The cephaloglycin dihydrate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Place an accurately measured representative portion of the suspension into an appropriate-sized volumetric flask and dilute to volume with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4). Further dilute an aliquot of the stock solution with solution 4 to the reference concentration of 10 micrograms of cephaloglycin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

(4) *Identity.* Dilute a representative portion of the sample with sufficient distilled water to give a concentration of 2.5 milligrams of cephaloglycin per milliliter (estimated). Shake vigorously on a mechanical shaker for 30 minutes. Filter through Whatman No. 1 filter paper, discarding the first few milliliters of filtrate. Further dilute an aliquot of the filtrate with sufficient distilled water to give a concentration of 0.05 milligram of cephaloglycin per milliliter (estimated). Using a suitable spectrophotometer, record the ultraviolet absorption spectrum of this solution from 230 to 320 nanometers. The spectrum compares qualitatively to that of the cephaloglycin working standard similarly treated.

§ 442.127 Cephalexin monohydrate oral dosage forms.

§ 442.127a Cephalexin monohydrate tablets.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Cephalexin monohydrate tablets are composed of cephalexin monohydrate and one or more suitable and harmless diluents, binders, lubricants, colorings, and coating substances. Each tablet contains cephalexin monohyrate equivalent to 500 milligrams of cephalexin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cephalexin that it is represented to contain. Its moisture content is not more than 9 percent. The tablets disintegrate within 30 minutes. The cephalexin monohydrate used conforms to the standards prescribed by § 442.27(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the

requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The cephalexin monohydrate used in making the batch for potency, safety, moisture, pH, absorptivity, identity, and crystallinity.

(b) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(a) The cephalexin monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of tablets into a high-speed glass blender jar containing sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Further dilute with solution 1 to the reference concentration of 20.0 micrograms of cephalexin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, preparing the sample as follows: Blend a representative number of tablets in a high-speed glass blender with sufficient distilled water to give a stock solution of convenient concentration. Further dilute with distilled water to the prescribed concentration of cephalexin.

NOTE: The 10.0 milliliters of 0.01*N* iodine must be added within 20 seconds after the addition of the 2.0 milliliters of 1.2*N* hydrochloric acid, and the assay should be completed within 1 hour after the sample and standard are first put into solution.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter, using the procedure described in paragraph (e) (1) of that section.

§ 442.127b Cephalexin monohydrate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cephalexin monohydrate capsules are composed of cephalexin monohydrate and one or more suitable and harmless lubricants and diluents enclosed in a gelatin capsule. Each capsule contains cephalexin monohydrate equivalent to either 125, 250, or 500 milligrams of cephalexin. Its potency is satisfactory if it is not less than 90 percent and not more the 120 percent of the number of milligrams of cephalexin that it is represented to contain. Its moisture content is not more than 10 percent. The cephalexin monohydrate used conforms to the standards prescribed by § 442.27 (a) (1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The cephalexin monohydrate used in making the batch for potency, safety, moisture, pH, absorptivity, identity, and crystallinity.

(b) The batch for potency and moisture.

(ii) Samples required:

(a) The cephalexin monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar with sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 1 to the reference concentration of 20.0 micrograms of cephalexin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, preparing the sample as follows: Blend a representative number of capsules in a high-speed glass blender with sufficient distilled water to give a stock solution of convenient concentration. Further dilute with distilled water to the prescribed concentration of cephalexin.

NOTE: The 10.0 milliliters of 0.01*N* iodine must be added within 20 seconds after the addition of the 2.0 milliliters of 1.2*N* hydrochloric acid, and the assay should be completed within 1 hour after the sample and standard are first put into solution.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 442.127c Cephalexin monohydrate for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cephalexin monohydrate for oral suspension is cephalexin monohydrate with one or more suitable and harmless diluents, buffer substances, colorings, and flavorings. When reconstituted as directed in the labeling, each milliliter contains cephalexin monohydrate equivalent to 25 milligrams, 50 milligrams, or 100 milligrams of cephalexin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cephalexin monohydrate that it is represented to contain. Its moisture content is not more than 2 percent. When reconstituted as directed in the labeling, its pH is not less than 3.0 and not more than 6.0. The cephalexin used conforms to the standards prescribed by § 442.27 (a) (1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The cephalexin used in making the batch for potency, safety, moisture, pH, absorptivity, identity, and crystallinity.

(b) The batch for potency, moisture, and pH.

(ii) Samples required:

(a) The cephalexin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Transfer an accurately measured representative portion of the suspension into an appropriate-sized volumetric flask and dilute to volume with 1-percent potassium phosphate buffer, pH 6.0 (solution 1). Further dilute an aliquot of this solution with solution 1 to the reference concentration of 20.0 micrograms of cephalexin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, preparing the sample as follows: Reconstitute the sample as directed in the labeling. Transfer an accurately measured representative portion to a volumetric flask and bring to volume with distilled water. Further dilute an aliquot of this solution with distilled water to the prescribed concentration of cephalexin.

NOTE: The 10 milliliters of 0.01*N* iodine must be added within 20 seconds after the addition of the 2.0 milliliters of 1.2*N* hydrochloric acid, and the assay should be completed within 1 hour after the sample and standard are first put into solution.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

Subpart C—Injectable Dosage Forms

§ 442.211 Sterile cefazolin sodium.

The requirements for certification and the tests and methods of assay for sterile cefazolin sodium packaged for dispensing are described in § 442.11a.

§ 442.223 Sterile cephaloridine.

The requirements for certification and the tests and methods of assay for sterile cephaloridine packaged for dispensing are described in § 442.23a.

§ 442.225 Sterile sodium cephalothin.

The requirements for certification and the tests and methods of assay for sterile sodium cephalothin packaged for dispensing are described in § 442.25a.

§ 442.229 Sterile cephapirin sodium.

The requirements for certification and the tests and methods of assay for sterile cephapirin sodium packaged for dispensing are described in § 442.29a.

PART 444—OLIGOSACCHARIDE ANTIBIOTIC DRUGS

Subpart A—Bulk Drugs

Sec.
444.10a Dihydrostreptomycin sulfate, crystalline dihydrostreptomycin sulfate, dihydrostreptomycin hydrochloride.
444.20 Gentamicin sulfate.
444.20a Sterile gentamicin sulfate.
444.30 Kanamycin sulfate.
444.30a Sterile kanamycin sulfate.
444.40 Neomycin palmitate.
444.42a Sterile neomycin sulfate.
444.42b [Editorial Note]
444.44 Neomycin undecylenate.
444.50 Paromomycin sulfate.
444.70a Streptomycin sulfate, streptomycin hydrochloride, streptomycin phosphate, streptomycin trihydrochloride calcium chloride (streptomycin calcium chloride complex).
444.72a Sterile streptonicozid sulfate.

Subpart B—Oral Dosage Forms

444.130 Kanamycin sulfate capsules.
444.142 Neomycin sulfate oral dosage forms.
444.142a Neomycin sulfate tablets.
444.142b Neomycin sulfate oral solution.
444.150 Paromomycin sulfate oral dosage forms.
444.150a Paromomycin sulfate capsules.
444.150b Paromomycin sulfate sirup.
444.170a Streptomycin-polymyxin-bacitracin tablets.

Subpart C—Injectable Dosage Forms

444.220 Gentamicin sulfate injection.
444.230 Kanamycin sulfate injection.
444.270 Streptomycin sulfate injectable dosage forms.
444.270a Sterile streptomycin sulfate, sterile streptomycin hydrochloride, sterile streptomycin phosphate, sterile streptomycin trihydrochloride calcium chloride (streptomycin calcium chloride complex).
444.270b Streptomycin sulfate injection.
444.270c Streptomycin sulfate for injection.

Subpart D—Ophthalmic Dosage Forms

444.320 Gentamicin sulfate ophthalmic dosage forms.
444.320a Gentamicin sulfate ophthalmic solution.
444.320b Gentamicin sulfate ophthalmic ointment.
444.342 Neomycin sulfate ophthalmic dosage forms.
444.342a Neomycin sulfate- _____ ophthalmic suspension; neomycin sulfate- _____ ophthalmic solution (the blanks being filled in with the established name(s) of the other active ingredient(s) present in accordance with paragraph (a)(1) of this section).
444.342b Neomycin sulfate-polymyxin B sulfate-gramicidin ophthalmic solution.
444.342c Neomycin sulfate- gramicidin- _____ ophthalmic solution; neomycin sulfate-gramicidin- _____ ophthalmic suspension (the blanks being filled in with the established name(s) of the other ingredient(s) present in accordance with paragraph (a)(1) of this section).
444.342d Neomycin sulfate-polymyxin B sulfate- _____ ophthalmic suspension (the blank being filled in with the established name(s) of the other active ingredient(s) present in accordance with paragraph (a)(1) of this section).
444.342e Neomycin sulfate ointment; neomycin sulfate- _____ ointment (the blank being filled in with the established name(s) of certain other active ingredient(s)).
444.342f Neomycin sulfate-gramicidin topical ointment; neomycin sulfate-gramicidin-triamcinolone acetonide ointment; neomycin sulfate -gramicidin - fludrocortisone acetate ointment.
444.342g Neomycin sulfate-hydrocortisone acetate eye-ear drops; neomycin sulfate-prednisolone acetate eye-ear drops.
444.342h Neomycin sulfate-polymyxin B sulfate ophthalmic ointment.
444.342i Neomycin sulfate-polymyxin B sulfate ophthalmic solution.

Subpart E—Otic Dosage Forms

444.442 Neomycin sulfate otic dosage forms.
444.442a Neomycin sulfate-sodium propionate otic solution; neomycin sulfate-sodium propionate-prednisolone acetate otic solution.
444.442b Neomycin sulfate-gramicidin-hydrocortisone-thonzylamine hydrochloride-thonzonium bromide otic solution.
444.442c Neomycin sulfate-polymyxin B sulfate-hydrocortisone otic suspension; neomycin sulfate-polymyxin B sulfate-hydrocortisone-sodium heparin otic suspension.
444.442d Neomycin sulfate ointment; neomycin sulfate- _____ ointment (the blank being filled in with the established name(s) of certain other active ingredient(s)).
444.442e Neomycin sulfate-hydrocortisone acetate eye-ear drops; neomycin sulfate-prednisolone acetate eye-ear drops.
444.470a Streptomycin otic with antifungal agent; streptomycin otic with _____; dihydrostreptomycin otic with antifungal agent; dihydrostreptomycin otic with _____ (the blank being filled in with the established name of the antifungal agent).

Subpart F—Dermatologic Dosage Forms

444.520 Gentamicin sulfate dermatologic dosage forms.
444.520a Gentamicin sulfate ointment.
444.520b Gentamicin sulfate cream.
444.540 Neomycin palmitate dermatologic dosage forms.
444.540a Neomycin palmitate-trypsin-chymotrypsin ointment.
444.540b Neomycin palmitate-trypsin-chymotrypsin concentrate lotion.
445.542 Neomycin sulfate dermatologic dosage forms.
444.542a Neomycin sulfate ointment; neomycin sulfate- _____ ointment (the blank being filled in with the established name(s) of the other active ingredient(s) present in accordance with paragraph (a)(1) of this section).
444.542b Neomycin sulfate- _____ cream (the blank being filled in with the established name(s) of the other active ingredient(s) present in accordance with paragraph (a)(1) of this section).
444.542c Neomycin sulfate- _____ lotion (the blank being filled in with the established name(s) of the other active ingredient(s) present in accordance with paragraph (a)(1) of this section).
444.542d Neomycin sulfate aerosol.
444.542e Neomycin sulfate-polymyxin B sulfate ointment.
444.542f Neomycin sulfate-gramicidin topical ointment; neomycin sulfate-gramicidin-triamcinolone acetonide ointment; neomycin sulfate-gramicidin - fludrocortisone acetate ointment.
444.542g Neomycin sulfate-gramicidin - triamcinolone acetonide cream.
444.542h Neomycin sulfate-gramicidin - triamcinolone acetonide lotion; neomycin sulfate - gramicidin - fludrocortisone acetate lotion.
444.542i Neomycin sulfate - triamcinolone acetonide topical aerosol; neomycin sulfate-dexamethasone topical aerosol.
444.542j Neomycin sulfate-polymyxin B sulfate-gramicidin-benzocaine ointment.
444.542k–444.542m [Editorial Note]
444.570 Streptomycin dermatologic dosage forms.
444.570a Streptomycin ointment; dihydrostreptomycin ointment.
444.570b Streptomycin for topical use; streptomycin with _____ (the blank being filled in with the name of the vehicle if a package combination) for topical use.
444.570c Streptomycin-bacitracin-polymyxin gauze pads.

Subpart G—Vaginal Dosage Forms

444.642a Neomycin sulfate-polymyxin B sulfate-acetarsone vaginal suppositories.

Subparts H and I—[Reserved]

Subpart J—Certain Other Dosage Forms

444.942 Neomycin sulfate in certain other dosage forms.
444.942a Neomycin sulfate for prescription compounding.
444.942b Sterile neomycin sulfate and polymyxin B sulfate solution.

AUTHORITY: Sec. 507, 59 Stat. 463, as amended (21 U.S.C. 357).

Subpart A—Bulk Drugs

§ 444.10a Dihydrostreptomycin sulfate, crystalline dihydrostreptomycin sulfate, dihydrostreptomycin hydrochloride.

(a) *Requirements for certification*—(1) Dihydrostreptomycin sulfate is the hydrogenated sulfate salt of a kind of streptomycin or a mixture of two or more such salts; crystalline dihydrostreptomycin sulfate is the hydrogenated crystalline sulfate salt of a kind of streptomycin or a mixture of two or more such salts; dihydrostreptomycin hydrochloride is the hydrogenated hydrochloride salt of a kind of streptomycin or a mixture of two or more such salts. Each such drug conforms to all requirements prescribed by § 444.70a(a) for streptomycin sulfate and streptomycin hydrochloride, and is subject to all procedures prescribed by 444.70a(a) for streptomycin sulfate and streptomycin hydrochloride, except that:

(i) Its potency is not less than 650 micrograms per milligram, except that if it is crystalline dihydrostreptomycin sulfate its potency is not less than 725 micrograms per milligram.

(ii) Its content of streptomycin sulfate or streptomycin hydrochloride is not more than 3.0 percent when calculated as streptomycin base, except that if it is crystalline dihydrostreptomycin

sulfate its content of streptomycin sulfate is not more than 1.0 percent.

(iii) Its labeling shall conform to the requirements of § 444.70a(a)(3)(iii).

(b) *Tests and methods of assay*—(1) *Potency.* Using the dihydrostreptomycin working standard as a standard of comparison, proceed as directed in § 444.70a(b)(1). Its potency is satisfactory if it contains not less than 90 percent of the number of milligrams that it is represented to contain.

(2) *Content of streptomycin sulfate or streptomycin hydrochloride*—(i) *Reagents.* (*a*) 10 percent ferric chloride stock solution. Dissolve 5 grams of $FeCl_3 6H_2O$ in 50 milliliters 0.1*N* HCl.

(*b*) 0.25 percent ferric chloride solution. Dilute 2.5 milliliters of 10 percent ferric chloride in 0.1*N* HCl to 100 milliliters with 0.01*N* MCl. Prepare the solution fresh daily.

(ii) *Standard curve.* Keep the working standard (obtained from the Food and Drug Administration) at −20° C. in tightly stoppered containers which in turn are kept in larger stoppered vials containing a suitable desiccant. Dry an appropriate amount of the working standard at 100° C. and a pressure of 5 millimeters or less for 4 hours. Prepare a stock aqueous solution containing 1.0 milligram of streptomycin base per milliliter. Store this standard solution in a refrigerator and use for no longer than 2 weeks. Transfer 1.0, 2.0, 3.0, 4.0, and 5.0 milliliters of this standard solution and 10 milliliters of distilled water to each of six 25-milliliter volumetric flasks. Add 9.0, 8.0, 7.0, 6.0, and 5.0 milliliters of distilled water to the five tubes, respectively, to give each a total volume of 10 milliliters. To each add 2.0 milliliters of 1*N* NaOH and then heat the flasks in a boiling water bath for 10 minutes. Cool the flasks in ice water for 3 minutes and acidify the solutions with 2.0 milliliters of 1.2*N* HCl. To each flask add 5.0 milliliters of 0.25 percent ferric chloride reagent, make to volume with distilled water, and mix thoroughly. Transfer the colored solutions to 2.0-centimeter absorption cells and measure the percent light transmission at 530 mμ in a suitable photoelectric colorimeter. Set the colorimeter at 100 percent light transmission for the zero concentration and then obtain the percent light transmission of the sample. Prepare a standard curve on semilog paper, plotting the percent light transmission on the logarithmic ordinate scale and the concentration of streptomycin base on the abscissa.

(iii) *Procedure.* Dilute the contents of a vial or a sufficient amount of bulk material to give a concentration of approximately 20 milligrams per milliliter. From the amount of streptomycin obtained, calculate the percent streptomycin as follows:

$$\text{Percent streptomycin} = \frac{\text{Milligrams of streptomycin} \times 100}{\text{Milligrams of dihydrostreptomycin found in the sample used}}$$

(3) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(4) *Toxicity, pyrogens, histamine, moisture, pH, crystallinity.* Proceed as directed in §§ 444.70a(b)(3), (4), (5), (6) and 440.80a(b)(5)(iii) of this chapter.

§ 444.20 Gentamicin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Gentamicin sulfate is the sulfate salt of a kind of gentamicin or a mixture of two or more such salts. It is a powder, white to buff in color. It is readily soluble in water but insoluble in ethanol. It is so purified and dried that:

(i) Its potency is not less than 590 micrograms of gentamicin per milligram on an anhydrous basis.

(ii) It passes the safety test.

(iii) Its loss on drying is not more than 18.0 percent.

(iv) Its pH in an aqueous solution containing 40 milligrams per milliliter is not less than 3.5 and not more than 5.5.

(v) Its specific rotation in an aqueous solution containing 10 milligrams per milliliter at 25° C. is not less than +107° and not more than +121°.

(vi) Its content of gentamicin C_1 is not less than 25 nor more than 50 percent; of gentamicin C_{1a}, not less than 15 nor more than 40 percent; and of gentamicin C_2, not less than 20 nor more than 50 percent.

(vii) It gives a positive identity test for gentamicin sulfate.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, loss on drying, pH, specific rotation, content of gentamicins C_1, C_{1a}, and C_2, and identity.

(ii) *Samples required.* 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute the stock solution with solution 3 to the reference concentration of 0.1 microgram of gentamicin per milliliter (estimated).

(2) *Safety.* Proceed as directed in § 436.33 of this chapter.

(3) *Loss on drying.* Proceed as directed in § 436.200(c) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 40 milligrams of gentamicin per milliliter.

(5) *Specific rotation.* Accurately weigh the sample to be tested in a volumetric flask and dilute with sufficient distilled water to give a solution containing approximately 10 milligrams per milliliter. Proceed as directed in § 436.210 of this chapter, using a 1.0-decimeter polarimeter tube and calculate the specific rotation on an anhydrous basis.

(6) *Content of gentamicins C_1, C_{1a}, and C_2.* Proceed as directed in § 444.20a(b)(8).

(7) *Identity.* Proceed as directed in § 436.211 of this chapter, using a 0.5 percent mixture of the sample in a potassium bromide disc prepared as described in paragraph (b)(1) of that section.

§ 444.20a Sterile gentamicin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile gentamicin sulfate is the sulfate salt of a kind of gentamicin or a mixture of two or more such salts. It is a powder, white to buff in color. It is readily soluble in water but insoluble in ethanol. It is so purified and dried that:

(i) Its potency is not less than 590 micrograms of gentamicin per milligram on an anhydrous basis.

(ii) It is sterile.

(iii) It passes the safety test.

(iv) It is nonpyrogenic.

(v) Its loss on drying is not more than 18.0 percent.

(vi) Its pH in an aqueous solution containing 40 milligrams per milliliter is not less than 3.5 and not more than 5.5.

(vii) Its specific rotation in an aqueous solution containing 10 milligrams per milliliter at 25° C. is not less than +107° and not more than +121°.

(viii) Its content of gentamicin C_1 is not less than 25 nor more than 50 percent; of gentamicin C_{1a}, not less than 15 nor more than 40 percent; and of gentamicin C_2, not less than 20 nor more than 50 percent.

(ix) It gives a positive identity test for gentamicin sulfate.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, safety, pyrogens, loss on drying, pH, specific rotation, content of gentamicins C_1, C_{1a}, and C_2, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute the stock solution with sufficient solution 3 to give a reference concentration of 0.1 microgram of gentamicin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Safety.* Proceed as directed in § 436.33 of this chapter.

(4) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 10.0 milligrams of gentamicin per milliliter.

(5) *Loss on drying.* Proceed as directed in §436.200(c) of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 40 milligrams of gentamicin per milliliter.

(7) *Specific rotation.* Accurately weigh the sample to be tested in a volumetric flask and dilute with sufficient distilled water to give a solution containing approximately 10 milligrams per milliliter. Proceed as directed in § 436.210 of this chapter, using a 1-decimeter polarimeter tube and calculate the specific rotation on an anhydrous basis.

(8) *Content of gentamicins C_1, C_{1a}, and C_2.*—(i) *Equipment*—(a) *Chamber (chromatographic).* Use a suitable chromatography jar with a tightly fitting, ground glass contact top for descending chromatography.

(b) *Sheets (chromatographic).* Cut a 57 x 46-centimeter sheet of Whatman No. 2 filter paper, or chromatographic paper that will produce similar results, into four strips of about 14.25 x 46 centimeters. Draw a starting line 9 centimeters from one end and mark two dots on this line, each 4 centimeters from each edge.

(ii) *Reagents.* Use reagent grade solvents and chemicals.

(iii) *Solvent system.* In each of two separators, equilibrate 200 milliliters of chloroform and 100 milliliters of methanol with 100 milliliters of 17 percent (9 molar) ammonium hydroxide. Without allowing the phases of one to separate, add the entire mixture to the chromatography jar and allow 24 hours for saturation. Allow the second separator to stand until the phases separate and use the lower phase only as the chromatographic solvent.

(iv) *Ninhydrin reagent.* To 1 gram of ninhydrin and 0.1 gram of cadmium acetate, add 3 milliliters of water and 1.5 milliliters of glacial acetic acid and shake. Add 100 milliliters of *n*-propanol and shake until solution is complete. Keep this solution in a brown bottle under refrigeration.

(v) *Procedure.* Prepare an aqueous solution containing 40 milligrams of the sample per milliliter. Apply 5 microliters of this solution to each dot on the sheet. Prepare two such sheets and place them in the tank so that elution will take place from separate troughs. Fill the two troughs with the chromatographic solvent. Develop the sheets in a descending manner until the solvent front reaches the bottom of the paper (approximately 3½ hours at 25° C.). Remove the sheets and dry in a hood for 30 minutes. Cut each sheet in half, lengthwise. Spray one half with ninhydrin reagent and place the sprayed strip in a drying oven at 100° C. for 1 minute. The gentamicin fractions appear as reddish zones. The zone furthest from the origin is gentamicin C_1, the one closest is gentamicin C_{1a}, and the middle zone is gentamicin C_2. Cut the corresponding zones out of the other unsprayed half of the sheet. Cut each portion of the sheet thus obtained into small strips and put those from each zone into a separate 125-milliliter glass-stoppered flask. Add 50 milliliters of 0.1*M* potassium phosphate buffer, pH 8, to each flask and swirl the flask mechanically for 30 minutes. Decant the solution from each flask into separate test tubes and allow the paper to settle. Pipet 4 milliliters of each clear solution into a 25-milliliter volumetric flask and make to volume with the pH 8 buffer. Assay these solutions as directed in paragraph (b)(1) of this section.

(vi) *Calculations.*

$$\text{Total gentamicins} = \frac{\text{Assay of } C_1 \text{ fraction}}{0.786} + \frac{\text{Assay of } C_2 \text{ fraction}}{1.023} + \frac{\text{Assay of } C_{1a} \text{ fraction}}{0.977}$$

$$\text{Percent of gentamicin } C_1 = \frac{\text{Assay of } C_1 \text{ fraction}}{0.786} \times \frac{100}{\text{total gentamicins}}$$

$$\text{Percent of gentamicin } C_2 = \frac{\text{Assay of } C_2 \text{ fraction}}{1.023} \times \frac{100}{\text{total gentamicins}}$$

$$\text{Percent of gentamicin } C_{1a} = \frac{\text{Assay of } C_{1a} \text{ fraction}}{0.977} \times \frac{100}{\text{total gentamicins}}$$

Where:

The assays are expressed in terms of the microgram equivalents of gentamicin; and The factors 0.786, 1.023, and 0.977 represent the activities of gentamicins C_1, C_2, and C_{1a} relative to the gentamicin activity of the gentamicin master standard.

(9) *Identity.* Proceed as directed in § 436.211 of this chapter, using a 0.5 percent mixture of the sample in a potassium bromide disc prepared as described in paragraph (b)(2) of that section.

§ 444.30 Kanamycin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Kanamycin sulfate is the sulfate salt of a kind of kanamycin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency on an anhydrous basis is not less than 750 micrograms of kanamycin per milligram.

(ii) It passes the safety test.

(iii) Its loss on drying is not more than 4 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 6.5 and not more than 8.5.

(v) Its residue on ignition is not more than 1.0 percent.

(vi) It gives a positive identity test for kanamycin.

(vii) It contains not more than 5.0 percent kanamycin B.

(viii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, loss on drying, pH, residue on ignition, identity, kanamycin B content, and crystallinity.

(ii) Samples required on the batch: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 10 micrograms of kanamycin per milliliter (estimated).

(2) *Safety.* Proceed as directed in § 436.33 of this chapter.

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution containing 10 milligrams per milliliter.

(5) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(6) *Identity.* Dissolve about 10 milligrams of kanamycin sulfate in 1 milliliter of water, and add 1 milliliter of a 1:500 solution of triketohydrindene hydrate in normal butyl alcohol; then add 0.5 milliliter of pyridine. Heat in a steam bath for 5 minutes and add 10 milliliters of water; a deep-purple color is produced.

(7) *Kanamycin B content*—(i) *Cylinders (cups).* Use cylinders described under § 440.80a(b)(1)(i) of this chapter.

(ii) *Culture medium.* Use ingredients that conform to the standards prescribed by the U.S.P. or N.F. Make agar for the base and seed layers as follows:

Peptone	6.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Agar	15.0 gm.
pH 7.8 to 8.0 after sterilization.	
Distilled water, q.s.	1,000.00 ml.

(iii) *Working standard.* Dissolve a suitable quantity of the kanamycin sulfate working standard, accurately weighed, in 0.1*M* potassium phosphate buffer, pH 8.0, to give a concentration equivalent to 1.0 milligram of kanamycin per milliliter.

(iv) *Preparation of sample.* To 100 milligrams, accurately weighed, of kanamycin sulfate in a suitable container (such as a 7.5-milliliter serum vial) add 5.0 milliliters of 6*N* hydrochloric acid,

and tightly close the container. Heat in a water bath at 100° C. for 1 hour and cool. Add 4 milliliters of 6*N* sodium hydroxide, then dilute with sterile 0.1*M* potassium phosphate buffer, pH 8.0, to obtain a concentration of the equivalent of 1 microgram of kanamycin per milliliter (estimated).

(v) *Preparation of test organism.* Use *Bacillus subtilis* (ATCC 6633)[1] prepared as described in § 436.103 of this chapter, using method 2.

(vi) *Preparation of plates.* Add 21 milliliters of the agar prepared as described in paragraph (b)(7) of this section to each Petri dish (20 millimeters x 100 millimeters). Distribute the agar evenly in the plates and allow to harden. Use the plates the same day they are prepared. Add 4.0 milliliters of the fresh daily inoculum described in paragraph (b)(7)(iv) of this section to each plate, tilting the plates back and forth to spread the inoculated agar evenly over the surface.

(vii) *Standard curve.* Prepare on the day of testing in 0.1M potassium phosphate buffer, pH 7.8 to 8.0, from the standard stock solution, sufficient volumes of the following concentrations: 0.64, 0.8, 1.0, 1.25, and 1.56 micrograms per milliliter. The 1.0 microgram-per-milliliter solution is the reference point of the standard curve. On each of three plates fill three cylinders with the 1.0 microgram-per-milliliter standard and the other three cylinders with the concentration under test. Thus, there will be thirty-six 1.0-microgram determinations for each of the other points on the curve. After the plates have incubated read the diameters of the circles of inhibition. Average the readings of the 1.0 microgram-per-milliliter concentration and the readings of the concentration test for each set of three plates and average also all 36 readings of the 1.0 microgram-per-milliliter concentration. The average of the 36 readings of the 1.0 microgram-per-milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 1.0 microgram-per-milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 0.8-microgram concentration, the average of the 36 readings of the 1.0 microgram-per-milliliter concentration is 16.5 millimeters and the average of the 1.0 microgram-per-milliliter concentration of this set of three plates is 16.3 millimeters, the correction is +0.2 millimeter. If the average readings of the 0.8 microgram-per-milliliter concentration of these same three plates is 15.9 millimeters, the corrected value is 16.1 millimeters. Plot these corrected values, including the average of the 1.0 microgram-per-milliliter concentration, on 2-cycle semilogarithmic paper, using the concentration in micrograms per milliliter as the ordinate and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points, either by inspection or by means of the following equations:

$$L = \frac{3a+2b+c-e}{5},$$

$$H = \frac{3e+2d+c-a}{5}$$

where:

L = Calculated zone diameter for the lowest concentration of the standard curve;

H = Calculated zone diameter for the highest concentration of the standard curve;

c = Average zone diameter of 36 readings of the 1.0 microgram-per-milliliter standard;

a, b, d, e = Corrected average values for the 0.64, 0.8, 1.0, 1.25, and 1.56 micrograms-per-milliliter solutions, respectively.

Plot the values obtained for L and H and connect with a straight line.

(viii) *Assay.* Place six cylinders on the inoculated agar surface in each Petri dish prepared as described in paragraph (b)(7)(vi) of this section, so that they are at approximately 60° intervals on a 2.8-centimeter radius. Use three plates for each sample. Fill three cylinders on each plate with the 1.0 microgram-per-milliliter standard and three cylinders with the 1.0 microgram (estimated)-per-milliliter sample, alternating standard and sample. Incubate plates for 16 hours to 18 hours at 32° C. to 35° C., and measure the diameter of each circle of inhibition.

(ix) *Estimation of kanamycin B content.* Average the zone readings of the standard and average the zone readings of the sample on the three plates used. If the sample gives larger average zone size than the average of the standard, add the difference between them to the 1.0-microgram zone size of the standard curve. If the average value is lower than the standard value, subtract the difference between them from the 1.0-microgram value on the curve. From the curve, read the kanamycin potencies corresponding to these corrected values of zone sizes. Multiply the observed potency by 100 and divide by 126 to obtain a value representing the potency in terms of the milligram equivalent of kanamycin B. The calculated amount of kanamycin B is not more than 5 percent of the content of kanamycin found in paragraph (b)(1) of this section.

(8) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

§ 444.30a Sterile kanamycin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Kanamycin sulfate is the sulfate salt of a kind of kanamycin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency on an anhydrous basis is not less than 750 micrograms of kanamycin per milligram.

(ii) It is sterile.

(iii) It passes the safety test.

(iv) It is nonpyrogenic.

(v) Its loss on drying is not more than 4 percent.

(vi) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 6.5 and not more than 8.5.

(vii) Its residue on ignition is not more than 1.0 percent.

(viii) It gives a positive identity test for kanamycin.

(ix) It contains not more than 5.0 percent kanamycin B.

(x) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, safety, pyrogens, loss on drying, pH, residue on ignition, identity, crystallinity, and kanamycin B content.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 10 micrograms of kanamycin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Safety.* Proceed as directed in § 436.33 of this chapter.

(4) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 10 milligrams of kanamycin per milliliter.

(5) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution containing 10 milligrams per milliliter.

(7) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(8) *Identity.* Dissolve about 10 milligrams of kanamycin sulfate in 1 milliliter of water and add 1 milliliter of a 1:500 solution of triketohydrindene hydrate in normal butyl alcohol. Then add 0.5 milliliter of pyridine. Heat in a steam bath for 5 minutes and add 10 milliliters of water; a deep-purple color is produced.

(9) *Kanamycin B content.* Proceed as directed in § 444.30(b)(7).

(10) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

§ 444.40 Neomycin palmitate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin palmitate is the palmitate salt of a kind of neomycin or a mixture of two or more such salts. It is so purified and dried that:

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

(i) It has a potency of not less than 180 micrograms of neomycin per milligram.

(ii) Its moisture content is not more than 5 percent.

(iii) The pH of a solution containing 50 milligrams per milliliter in 80 percent methyl alcohol is not less than 6.5 and not more than 7.5.

(iv) It exhibits a positive identity test for neomycin and meets a specified melting range for palmitic acid which is derived from the palmitate salt.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter. Its expiration date is 12 months.

(3) *Requests for certification.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, and identity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 444.42a(b)(1)(i), except prepare the sample in the following manner: Blend an accurately weighed sample of approximately 30 milligrams in 19.0 milliliters of methyl alcohol plus 1.0 milliliter of polysorbate 80. Use a high-speed glass blender. Transfer dropwise a 1.0 milliliter aliquot into that volume of 0.1*M* potassium phosphate buffer, pH 8.0, which will give a final (estimated) concentration of 1.0 microgram of neomycin per milliliter.

(2) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) of this chapter.

(3) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, except prepare a solution containing 50 milligrams per milliliter in 80 percent methyl alcohol.

(4) *Identity*—(i) *Neomycin.* Add 1 gram of neomycin palmitate to 50 milliliters of methyl alcohol in a 100-milliliter beaker. Stir, and heat carefully to about 35° C. to dissolve. After the neomycin palmitate is in solution, carefully adjust the solution to pH 5.8±0.2 by the dropwise addition of sulfuric acid. Stir the solution while adding the acid. Allow to stand one-half hour. Filter off the precipitate and wash it with 5–8 milliliters of methyl alcohol. Save the entire methyl alcohol filtrate. Dry the precipitate thoroughly in an oven not exceeding 60° C. After drying, powder the material if necessary, transfer about 10 milligrams to a test tube (19 millimeters x 150 millimeters) and dissolve in 1 milliliter of water, and add 5 milliliters of 40 percent sulfuric acid solution. Heat in a boiling water bath 100 minutes. Allow to cool. Dilute to a volume of 25 milliliters with distilled water. Adjust a suitable recording ultraviolet spectrophotometer to zero absorbance from 220 mμ to 300 mμ, using an 8 percent sulfuric acid solution and 1-centimeter cells. Record the spectrum of the sample solution between these wavelengths. The ratio of the two absorbances $\frac{(A278)}{(A232)}$ should not be less than 1.20 and not more than 1.50.

(ii) *Palmitate.* Evaporate to dryness the methyl alcohol filtrate obtained from the procedure described in paragraph (b)(4)(i) of this section. Dry the solid in vacuum for 2 hours at 40° C. Determine the melting point of the dried material. The melting point is not less than 57.0° C. nor more than 61.0° C. This indicates the purity of palmitic acid derived from neomycin palmitate.

§ 444.42a Sterile neomycin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate is the sulfate salt of a kind of neomycin or a mixture of two or more such salts. It is so purified and dried that:

(i) It has a potency of not less than 600 micrograms of neomycin per milligram, calculated on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It is nontoxic.

(v) Its moisture content is not more than 8.0 percent.

(vi) Its pH in an aqueous solution containing 33 milligrams per milliliter is not less than 5.0 and not more than 7.5.

(vii) It gives a positive identity test for neomycin.

(2) *Packaging.* In addition to the requirements of § 432.1 of this chapter, each immediate container shall contain 0.35 gram of neomycin.

(3) *Labeling.* In addition to the requirements of § 432.5 of this chapter, if the drug is packaged for dispensing its labeling shall bear adequate warnings for its intramuscular or intraperitoneal use for hospitalized patients only, so as to provide constant supervision by a physician. The labeling shall bear the statement "Refrigerate solution". Its expiration date is 12 months.

(4) *Request for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, toxicity, moisture, pH, and identity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in the manufacture of another drug.

(*1*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 12 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods:

(i) *Plate assay using Staphylococcus epidemmidis (ATCC 12228)*[1] (a) *Cylinders (cups).* Use cylinders described in § 440.80a(b)(1)(i) of this chapter.

(*b*) *Culture media.* Using ingredients that conform to the standards prescribed by the U.S.P. or N.F.:

(*1*) Make nutrient agar for carrying the test organism as follows:

Peptone	6.0 gm.
Pancreatic digest of casein	4.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Dextrose	1.0 gm.
Agar	15.0 gm.
Distilled water q.s.	1,000.0 ml.

pH 6.5 to 6.6 after sterilization.

(*2*) Make nutrient agar for the base and seed layers as described in paragraph (b)(1)(i)(*b*)(*1*) of this section, except that its pH after sterilization is 7.8 to 8.0.

In lieu of preparing the media from the individual ingredients specified in paragraph (b)(1)(i)(*b*) of this section, they may be made from a dehydrated mixture that, when reconstituted with distilled water, has the same composition as such media. Minor modification of the individual ingredients specified in paragraph (b)(1)(i)(*b*) of this section are permissible if the resulting media possess growth-promoting properties at least equal to the media described.

(*c*) *Working standard.* Dry a portion of the working standard for 3 hours at 60° C. and a pressure of 5 millimeters or less. Determine the dry weight, and dissolve in sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a stock solution of convenient concentration. When stored under refrigeration, the stock solution may be used for a period not exceeding 2 weeks.

(*d*) *Preparation of sample.* Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a stock solution of convenient concentration; and also, if it is packaged for dispensing, reconstitute as directed in the labeling, remove an accurately measured representative portion with a suitable syringe fitted with a 22-gage needle; and dilute with sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a stock solution of convenient concentration. Further dilute the stock solution with sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(*e*) *Preparation of test organism.* The test organism is *Staphylococcus epidermidis* (ATCC 12228),[1] which is maintained on slants of nutrient agar described in (b)(1)(i)(*b*)(*1*) of this section. Using 3 milliliters of U.S.P. saline T.S., wash the organism from the nutrient agar slant (which has been incubated for 24 hours at 32° C.–35° C.) onto a large nutrient agar surface such as that provided by a Roux bottle containing

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

300 milliliters of nutrient agar. Incubate for 24 hours at 32° C.–35° C. Wash the resulting growth from the nutrient surface, using 50 milliliters of sterile U.S.P. saline T.S. Adjust the volume of the suspension so that a 1:14 dilution will give 25 percent light transmission when measured with a suitable photoelectric colorimeter having a 580 mμ filter and a 13-millimeter diameter test tube as an absorption cell. By the use of test plates, determine the appropriate inoculum of the adjusted suspension (usually 0.1 milliliter) to be inoculated to each 100 milliliters of seed layer agar in order to obtain satisfactory zones of inhibition. The suspension may be used for 1 week if stored under refrigeration.

(*f*) *Preparation of plates.* Add 21 milliliters of the agar prepared as described in paragraph (b) (1) (i) (*b*) (2) of this section to each Petri dish (20 millimeters x 100 millimeters). Distribute the agar evenly in the plates and allow to harden on a level surface. Accurately measure a sufficient quantity of the nutrient agar, cool to 48° C., and add the appropriate inoculum of the adjusted suspension, prepared as described in paragraph (b) (1) (i) (*e*) of this section. Swirl the inoculated nutrient agar to obtain a homogeneous suspension, and add 4 milliliters to each of the plates containing the 21 milliliters of uninoculated nutrient agar. Tilt the plates back and forth to spread the inoculated nutrient agar evenly, and allow to harden on a level surface. After the agar has hardened, place six cylinders described in paragraph (b) (1) (i) (*a*) of this section on the inoculated agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius. Use the plates the same day they are prepared.

(*g*) *Standard curve.* Using the stock solution of the working standard prepared as described in paragraph (b) (1) (i) (*c*) of this section, prepare solutions in 0.1*M* potassium phosphate buffer pH 8.0 of the following concentrations: 0.64, 0.8, 1.0, 1.25, 1.56 micrograms of neomycin per milliliter. The 1.0 microgram per milliliter concentration is the reference concentration of the assay. Use a total of 12 plates, three plates for each solution except the reference point solution which is included on each plate. On each of the three plates, fill three cylinders with the reference point solution and the other three cylinders with the concentrations under test. Thus, there will be 36 reference point determinations and nine determinations for each of the other points on the curve. After the plates have incubated, read the diameters of the circles of inhibition. Average the readings of the reference point concentration and the readings of the point tested for each set of three plates and average also all 36 readings of the reference point concentration. The average of the 36 readings of the reference point concentration is the correction point of the curve. Correct the average value obtained for each point to the figure it would be if the reference point reading for that set of three plates were the same as the correction point. Thus, if in correcting the 0.8-microgram concentration, the average of the 36 readings of the 1.0 microgram per milliliter (reference point) concentration is 16.5 millimeters and the average of the 1.0 microgram per milliliter concentration of the set of three plates (the 0.8 microgram per milliliter set) is 16.3 millimeters, the correction is +0.2 millimeter. If the average readings of the 0.8 microgram per milliliter concentration of these same three plates is 15.9 millimeters, the corrected value is then 16.1 millimeters. Plot these corrected values, including the average of the 1.0 microgram per milliliter concentration, on 2-cycle semilog paper, using the concentration in micrograms per milliliter as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points either by inspection or by means of the following equations:

$$H=\frac{3a+2b+c-e}{5},$$

$$L=\frac{3e+2d+c-a}{5},$$

where:

L = Calculated zone diameter for the lowest concentration of the standard curve;

H = Calculated zone diameter for the highest concentration of the standard curve;

c = Average zone diameter of 36 readings of the reference point standard solution;

a, b, d, e = Corrected average values for the other standard solutions, lowest to highest concentrations, respectively.

(*h*) *Assay procedure.* Use three plates for each sample. Fill three cylinders on each plate with the standard and three cylinders with the sample, which has been diluted to the reference concentration, alternating standard and sample. Incubate the plates for 16 hours to 18 hours at 32° C.–35° C., and then measure the diameter of each zone of inhibition. To estimate the potency of the sample, average the zone readings of the standard and the zone readings of the sample on the three plates used. If the sample gives a larger zone size than the average of the standard, add the difference between them to the reference point zone of the standard curve. If the average value is lower than the standard value, subtract the difference between them from the reference point value on the curve. From the curve, read the potencies corresponding to these corrected values of zone sizes.

(ii) *Plate assay using Staphylococcus aureus (ATCC 6538P)*[1] Proceed as directed in paragraph (b) (1) (i) of this section, except that the reference concentration of the sample under test is 10.0 micrograms of neomycin per milliliter; the concentrations of the standard curve solutions are 6.4, 8.0, 10.0, 12.5, 15.6 micrograms of neomycin per milliliter; and the suspension of the test organism, *Staphylococcus aureus* (ATCC 6538P),[1] is adjusted so that a 1:19 dilution will give 25 percent light transmission and the usual inoculum for each 100 milliliters of agar for the seed layer is 0.2 milliliter of the diluted suspension.

If it is packaged for dispensing, its content of neomycin is not less than 90 percent and not more than 120 percent of the number of milligrams of neomycin that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e) (1) of that section.

(3) *Pyrogens.* Proceed as directed in § 440.80a(b) (3) of this chapter, using a test dose of 1.0 milliliter per kilogram of a solution containing 10 milligrams of neomycin per milliliter in pyrogen-free, sterile U.S.P. saline T.S.

(4) *Toxicity.* Proceed as directed in § 440.80a(b) (4) of this chapter, using 0.5 milliliter of a solution containing 200 micrograms of neomycin per milliliter in sterile U.S.P. saline T.S.

(5) *Moisture.* In an atmosphere of about 10 percent relative humidity, transfer about 100 milligrams of the finely powdered sample to a tared weighing bottle equipped with ground-glass top and stopper. Weigh the bottle and place it in a vacuum oven, tilting the stopper on its side so that there is no closure during the drying period. Dry at a temperature of 60° C. and a pressure of 5 millimeters of mercury or less for 3 hours. At the end of the drying period, fill the vacuum oven with air dried by passing it through a drying agent such as sulfuric acid or silica gel. Replace the stopper and place the weighing bottle in a desiccator over a desiccating agent such as phosphorus pentoxide or silica gel, allow to cool to room temperature, and reweigh. Calculate the percent loss.

(6) *pH.* Proceed as directed in § 440.80a(b) (5) (ii) of this chapter, using a solution containing 33 milligrams of neomycin per milliliter.

(7) *Identity*—(i) *Reagents.* (*a*) Sulfuric acid solution: Mix concentrated sulfuric acid and distilled water in volumetric proportions of 40:60.

(*b*) Xylene.

(*c*) *p*-Bromoaniline: (Prepare and store this reagent in brown, nonactinic glassware.) Place 380 milliliters of thioureasaturated glacial acetic acid solution in the bottle, add 10 milliliters of 20 percent sodium chloride solution, 5 milliliters of 5 percent oxalic acid solution, and 5 milliliters of 10 percent disodium phosphate solution, and mix well. Add 8 grams of *p*-bromoaniline and mix well. Let this reagent stand overnight before use. Prepare the reagent once weekly.

(ii) *Procedure.* Place about 10 milligrams of the sample into a test tube (19 millimeters x 150 millimeters), dissolve with 1 milliliter of water, and then carefully add 5 milliliters of the sulfuric acid solution. Heat in a boiling water bath for 100 minutes. Cool to room temperature. Add 10 milliliters of xylene to the test tube. Stopper the tube and shake vigorously for about 1 minute. Let the two layers separate and then decant the xylene layer into a second test tube. Add 10 milliliters of the *p*-bromoaniline reagent to the xylene solution, shake, and let stand. The development of a vivid pink-red color is a positive identity test for neomycin.

EDITORIAL NOTE: § 444.42b (formerly § 1481.31) was revoked at 37 FR 25820,

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

Dec. 5, 1972, to become effective in 40 days. Objections and a request for hearing were received by the Commissioner of Food and Drugs.

Pending a review of the objections, the Commissioner has determined that the effective date of the revocation should be postponed. See 38 FR 7007, Mar. 15, 1973. For convenience of the user the revoked section is set forth below in its entirety.

§ 444.42b Neomycin sulfate (commercial grade).

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate (commercial grade) is the sulfate salt of a kind of neomycin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 400 micrograms of neomycin per milligram on the anhydrous basis.

(ii) It passes the toxicity test.

(iii) Its moisture content is not more than 8.0 percent.

(iv) Its pH in an aqueous solution containing 33 milligrams of neomycin sulfate commercial grade per milliliter is not less than 4.0 and not more than 7.5.

(v) It gives a positive identity test for neomycin sulfate.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter. Its expiration date is 12 months.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, toxicity, moisture, pH, and identity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 444.42a(b)(1).

(2) *Toxicity.* Proceed as directed in § 444.42a(b)(4).

(3) *Moisture.* Proceed as directed in § 444.42a(b)(5).

(4) *pH.* Proceed as directed in § 444.42a(b)(6).

(5) *Identity.* Proceed as directed in § 444.42a(b)(7).

§ 444.44 Neomycin undecylenate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin undecylenate is the undecylenate salt of a kind of neomycin or a mixture of two or more such salts. It is so purified and dried that:

(i) It contains not less than 300 micrograms of neomycin per milligram, calculated on an anhydrous basis.

(ii) It contains not less than 600 micrograms of undecylenic acid per milligram, calculated on an anhydrous basis.

(iii) Its moisture content is not more than 5.0 percent.

(iv) The pH of a solution containing 50 milligrams per milliliter in 80 percent methyl alcohol is not less than 6.5 and not more than 7.0.

(v) It gives a positive identity test for neomycin undecylenate.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency of neomycin and undecylenic acid, moisture, pH, and identity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Neomycin content.* Proceed as directed in § 444.42a(b)(1)(i), except prepare the sample for assay as follows: Accurately weigh aproximately 500 milligrams of neomycin undecylenate, transfer to a 250-milliliter volumetric flask, add 50 milliliters of chloroform, and mix well. Adjust to volume with 95 percent ethyl alcohol, and mix well. Dilute an aliquot with 0.1*M* potassium phosphate buffer, pH 8.0, to the reference concentration of 1.0 microgram of neomycin per milliliter.

(ii) *Undecylenic acid content*—(a) *Reagents*—(1) *Alcohol.* Use 95 percent ethyl alcohol (aldehyde-free).

(2) *Phenolphthalein indicator.* Dissolve 1 gram of phenolphthalein in 100 milliliters of alcohol.

(3) *Alcoholic sodium hydroxide (about 0.1N).* Prepare alcoholic sodium hydroxide solution as follows: Weigh rapidly about 5 grams of sodium hydroxide pellets, dissolve in 10 milliliters of water, and add sufficient alcohol to make 1,000 milliliters. Allow the solution to stand in a tightly stoppered bottle protected from light for 24 hours. Then quickly decant the clear supernatant liquid into a suitable tight container and store protected from light. Determine the undecylenic acid equivalent of this solution each time an assay is performed.

(b) *Procedure.* Weigh accurately two 100-milligram portions of undecylenic acid (N.F. grade) and place each into separate Erlenmeyer flasks. Also make two accurate weighings of the neomycin undecylenate sample, each consisting of about 120 milligrams, and place each into separate Erlenmeyer flasks. Carefully dissolve each weighing in 10 milliliters of alcohol. Add two drops of phenolphthalein indicator and titrate to the endpoint. Determine the amount of alcoholic sodium hydroxide equivalent to 10 milliliters of alcohol and make this reagent-blank correction for each titration. Determine the undecylenic acid equivalent of the alcoholic sodium hydroxide, and calculate the undecylenic acid content of the neomycin undecylenate sample.

(2) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) of this chapter.

(3) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, except use a solution containing 50 milligrams per milliliter in 80 percent methyl alcohol.

(4) *Identity.* Weigh approximately 1 milligram of neomycin undecylenate and mix thoroughly with 200 milligrams of dried infrared spectrophotometric quality potassium bromide in a small ball mill for about 1 minute. Prepare a potassium bromide disc by transferring the uniform mixture to a die of suitable size. Evacuate gradually while raising the pressure to 3,000 pounds per square inch. Hold at 3,000 pounds per square inch until evacuation is complete and then raise the pressure to 20,000 pounds per square inch. Hold this pressure for 2 or 3 minutes. Using a suitable infrared spectrophotometer, run the spectrum from 2 μ to 16 μ. Use an attenuator if necessary. Characteristic undecylenic acid peak absorbances occur at 7.1 and 11 μ distinguishing neomycin undecylenate from neomycin sulfate.

§ 444.50 Paromomycin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Paromomycin sulfate is the sulfate salt of a kind of paromomycin or a mixture of two or more such salts. It is a creamy-white to light-yellow powder. It is so purified and dried that:

(i) Its potency is not less than 675 micrograms per milligram on an anhydrous basis.

(ii) It passes the safety test.

(iii) Its loss on drying is not more than 5.0 percent.

(iv) The pH of a 3.0 percent aqueous solution is not less than 5.0 and not more than 7.5.

(v) Its specific rotation at 25° C. in water is not less than +50° and not more than +55° on an anhydrous basis.

(vi) Its residue on ignition is not more than 2.0 percent.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, loss on drying, pH, specific rotation, and residue on ignition.

(ii) Samples of the batch: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute the stock solution with solution 3 to the reference concentration of 1.0 microgram of paromomycin per milliliter (estimated).

(2) *Safety.* Proceed as directed in § 436.33 of this chapter.

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a 3.0 percent aqueous solution.

(5) *Specific rotation.* Accurately weigh approximately 1.25 grams of the sample into a 25-milliliter volumetric flask. Dissolve in a few milliliters of water, add water to volume, and mix. Proceed as directed in § 436.210 of this chapter, using a 2.0-decimeter polarimeter tube. Calculate the specific rotation on an anhydrous basis.

(6) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

§ 444.70a Streptomycin sulfate, streptomycin hydrochloride, streptomycin phosphate, streptomycin trihydrochloride calcium chloride (streptomycin calcium chloride complex).

(a) *Requirements for certification*—(1) *Standards of identity, strength,*

quality, and purity. Streptomycin sulfate is the sulfate salt of a kind of streptomycin or a mixture of two or more such salts; streptomycin hydrochloride is the hydrochloride salt of a kind of streptomycin or a mixture of two or more such salts; streptomycin phosphate is the phosphate salt of a kind of streptomycin or a mixture of two or more such salts; streptomycin trihydrochloride calcium chloride is the double salt of a kind of streptomycin or a mixture of two or more such salts. Each such drug is so purified and dried that:

(i) Its potency is not less than 650 micrograms per milligram;

(ii) It is sterile;

(iii) It is nontoxic;

(iv) It is nonpyrogenic;

(v) It contains no histamine or histamine-like substances;

(vi) Its moisture content is not more than 5.0 percent;

(vii) Its pH in aqueous solution of 0.2 gram per millimeter is not less than 4.5 and not more than 7.0.

(2) *Packaging.* In all cases the immediate container shall be a tight container as defined by the U. S. P., shall be sterile at the time of filling and closing, shall be so sealed that the contents cannot be used without destroying the seal, and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded. In case it is packaged for dispensing, it shall be in immediate containers of colorless transparent glass closed by a substance through which a hypodermic needle may be introduced and withdrawn without removing the closure or destroying its effectiveness. Each such container shall contain 0.5 gram, 1.0 gram, 2.0 grams, 3.0 grams, 4.0 grams, 5.0 grams, or 10.0 grams, and each may be packaged in combination with a container of the solvent water for injection U. S. P., dextrose injection 5 percent U. S. P. or physiological salt solution U.S.P.

(3) *Labeling*—(i) *It is packaged for dispensing.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its label or labeling, as hereinafter indicated, the following:

(*a*) On the outside wrapper or container and the immediate container, the statement, "Expiration date _____", the blank being filled in with the date that is 48 months after the month during which the batch was certified, except that the blank may be filled in with the date that is 60 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such a period of time such drug as prepared by him complies with the standards prescribed by paragraph (a)(1) of this section.

(*b*) On the circular or other labeling within or attached to the package, if it is packaged for dispensing, the conditions under which such solutions should be stored, including the statement, "Sterile solutions may be stored at room temperature for 4 weeks without significant loss of potency".

(ii) *It is packaged solely for manufacturing use and/or repacking.* Each package shall bear on its outside wrapper or container and the immediate container the following:

(*a*) The potency per gram and the number of grams in the immediate container.

(*b*) The statement "Caution: Federal law prohibits dispensing without prescription".

(c) The statement "For manufacturing use", "For repacking", or "For manufacturing use or repacking".

(*d*) The information required by paragraph (a)(3)(i)(*a*) of this section.

(4) *Request for certification, check tests and assays; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in the batch, the number of grams in each package, and (unless it was previously submitted) the date on which the latest assay of the drug comprising the batch was completed. Such request shall be accompanied or followed by the results of tests and assays made by him on the batch for potency, sterility, toxicity, pyrogens, histamine content (except that if the batch is packaged for dispensing, the result of this test performed on the streptomycin sulfate used in filling the batch may be submitted instead), moisture, pH, and identity. If such batch or any part thereof is to be packaged with a solvent, such request shall also be accompanied by a statement that such solvent conforms to the requirements prescribed therefor by this section.

(ii) If such batch is packaged for dispensing, such person shall submit with his request an accurately representative sample of the batch, consisting of the following:

(*a*) For all tests except sterility: One immediate container for each 5,000 immediate containers in such batch, but in no case less than 12 immediate containers.

Such samples shall be collected by taking single immediate containers at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(*b*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(iii) If such batch is packaged for repacking or for use as an ingredient in the manufacture of another drug, such person shall submit with his request an accurately representative sample of the batch, consisting of the following:

(*a*) For all tests except sterility: 10 packages.

(*b*) For sterility testing: 20 packages.

Each such package shall contain approximately 0.5 gram taken from a different part of such batch, and each shall be packaged in accordance with the requirements of paragraph (a)(2) of this section.

(iv) In connection with contemplated requests for certification of repackaged batches or batches of another drug in the manufacture of which it is to be used, the manufacturer of a batch which is to be so repacked or used may request the Commissioner to make check tests and assays on a sample of such batch taken as prescribed by paragraph (a)(3) of this section. From the information required by paragraph (a)(4)(i) of this section may be omitted results of tests and assays not required for the batch when used in such other drug. The Commissioner shall report to such manufacturer results of such check tests and assays as are so requested.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Cylinders (cups).* Use cylinders described under § 440.80a(b)(1)(i) of this chapter.

(ii) *Culture media.* Using ingredients that conform to the standards prescribed by the U.S.P. or N.F., make nutrient agar for the seed and base layers:

Beef extract	1.5 gm.
Yeast extract	3.0 gm.
Peptone	6.0 gm.
Agar	15.0 gm.
Distilled water q. s.	1,000.0 ml.

pH 7.8 to 8.0 after sterilization.

(iii) *Working standard.* Keep the working standard (obtained from the Food and Drug Administration) at −20° C. in tightly stoppered containers which in turn are kept in larger stoppered vials containing a suitable desiccant. Dry an appropriate amount of the working standard as described in § 440.80a(b)(5)(i) of this chapter. Dissolve the weight of the dry working standard obtained in distilled water. Keep this stock solution in a refrigerator. Do not use it later than 30 days after it is made.

(iv) *Standard curve.* Prepare daily in 0.10*M* potassium phosphate buffer, pH 8.0, from the stock solution described in paragraph (b)(1)(iii) of this section, concentrations of 0.64, 0.80, 1.0, 1.25, and 1.56 micrograms per milliliter solution. A total of 12 plates is used in the preparation of the standard curve, three plates for each solution except the 1.0 microgram per milliliter solution. The latter concentration is used as the reference point and is included on each plate. On each of three plates fill three cylinders with the 1.0 microgram per milliliter standard and the other three cylinders with the concentration under test. Thus there will be thirty-six 1-microgram determinations and nine determinations for each of the other points on the curve. After the plates have incubated, read the diameters of the circles of inhibition. Average the readings of the 1.0 microgram per milliliter concentration and the readings of the point tested for each set of three plates and averages also all 36 readings of the 1.0 microgram per milliliter concentration. The average

of the 36 readings of the 1.0 microgram per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 1.0 microgram per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 0.8-unit concentration, the average of the 36 readings of the 1.0 microgram per milliliter concentration is 16.5 millimeters and the average of the 1.0 microgram per milliliter concentration of this set of three plates is 16.3 millimeters, the correction is 0.2 millimeter. If the average readings of the 0.8 microgram per milliliter concentration of these same there plates is 15.9 millimeters, the corrected value is then 16.1 millimeters. Plot these corrected values, including the average of the 1.0 microgram per milliliter concentration, on 2-cycle semilog paper using the concentration in micrograms per milliliter as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points, either by inspection or by the equations in § 440.80a(b)(1)(viii) of this chapter. The five points selected to determine the curve are arbitrary (they should be equally spaced) and should be so chosen that the limits of the curve will fill the needs of the laboratory. However, the potency of the sample under test should fall in the interval of from 64 percent to 156 percent of the correction point of the standard curve.

(v) *Preparation of sample.* Dissolve the sample to be tested in sufficient 0.1 *M* potassium phosphate buffer, pH 8.0, to make a convenient stock solution. Further dilute in buffer to a final concentration of 1.0 microgram per milliliter (estimated). Use this last dilution in the assay for potency.

(vi) *Preparation of suspensions—(a) Preparation of spore suspensions.* The test organism is *Bacillus subtilis* (American Type Culture Collection 6633).[1] Maintain the test organism on nutrient agar prepared as described in § 440.80a(b)(1)(ii)(*a*) of this chapter. Prepare a spore suspension by one of the following methods:

(*1*) Grow the organism for 1 week at 37° C. in a number of Roux bottles each containing 300 milliliters of nutrient agar prepared as described in § 440.80a(b)(1)(ii)(*a*) of this chapter. Suspend the spores in sterile distilled water and heat for 30 minutes at 65° C. Wash the spore suspension three times with sterile distilled water, heat again for 30 minutes at 65° C. and resuspend in sterile distilled water; or

(*2*) Grow the organism for 5 days at 37° C. in a Roux bottle containing 300 milliliters of agar medium described in § 440.80a(b)(1)(ii)(*a*) of this chapter, but modified by the addition of 300 milligrams of $MnSO_4 \cdot H_2O$ per liter. Suspend the growth in 50 milliliters of sterile isotonic saline solution, centrifuge, and decant the supernatant liquid. Reconstitute the sediment and heat-shock the suspension by heating for 30 minutes at 70° C. Maintain the spore suspension at approximately 15° C. Determine by appropriate tests the quantity of spore suspension to be added to each 100 milliliters of agar for the secondary layer that will give sharp, clear zones of inhibition.

(*b*) *Preparation of vegetative suspension.* If a suspension of the test organism is to be used in lieu of the spore suspensions described in paragraph (b)(1)(vi)(*a*) of this section, prepare such suspension as follows: Using 2.0 milliliters of sterile distilled water, wash the organism from an agar slant (which has been incubated for 24 hours at 37° C.) onto the agar surface of a Roux bottle containing 300 milliliters of nutrient agar prepared as described in § 440.80a(b)(1)(ii)(*a*) of this chapter. Incubate for 24 hours at 37° C. Suspend the resultant growth in 50 milliliters of sterile distilled water and homogenize the suspension. Determine by appropriate tests the quantity of the vegetative suspension to be added to each 100 milliliters of agar for the secondary layer that will give sharp, clear zones of inhibition.

(vii) *Preparation of plates.* Add 21 milliliters of agar described in paragraph (b)(1)(ii) of this section to each Petri dish (20 by 100 millimeters). Melt the agar to be used for the secondary layer, cool to 55° to 60° C. and add the spore suspension prepared in paragraph (b)(1)(vi) of this section. Mix thoroughly and add 4 milliliters to each of the plates containing the 21 milliliters of the uninoculated agar. Tilt the plates back and forth to spread the inoculated agar evenly over the surface.

(viii) *Plate assay.* Place six cylinders on the inoculated agar surface so that they are at approximately 60° intervals on 2.8-centimeter radius. Use three plates for each sample. Fill three cylinders on each plate with the 1.0 mcg./ml. standard and three cylinders with the 1.0 mcg./ml. (estimated) sample, alternating standard and sample. Incubate the plates for 16 to 18 hours at 37° C. and measure the diameter of each circle of inhibition.

(ix) *Estimation of potency.* Average the zone readings of the standard and average the zone readings of the sample on the three plates used. If the sample gives a larger average zone size than the average of the standard, add the difference between them to the 1.0 microgram zone size of the standard curve. If the average sample value is lower than the standard value, subtract the difference between them from the 1.0 microgram value on the curve. From the curve read the potencies corresponding to these corrected values of zone sizes.

(x) *Turbidimetric assay.* In lieu of the plate-assay method described in paragraph (b)(1)(viii) of this section, the sample may be assayed for potency by the following method:

(*a*) Employ the agar described in paragraph (b)(1)(ii) of this section (adjusted to a final pH of 7.0) for maintaining the test organism, which is *Klebsiella pneumoniae* (ATCC 10031),[1] noncapsulated. Transfer stock cultures every week for test purposes. Transfer the organism to fresh agar slants and incubate overnight at 37° C. Suspend the growth from two or three of these slants in sterile distilled water and add approximately 5 milliliters of culture suspension to each of two Roux bottles containing the agar described in paragraph (b)(1)(ii) of this section. Incubate the bottles overnight at 37° C., harvest the growth, using 50 milliliters of sterile distilled water per bottle, and pool the washings from the two bottles. Determine the dilution with water that will give a light transmission reading of 65 percent, using a filter of 6,500 Angstrom units in a photoelectric colorimeter. Keep the resulting suspension of organisms in the refrigerator and use for a period of not to exceed 1 week. Prepare a daily inoculum by adding 6.0 milliliters of the diluted suspension to each 100 milliliters of the nutrient broth prepared as directed in § 440.80a(b)(1)(ii)(*c*) of this chapter, cooled to a temperature of approximately 15° C.

(*b*) *Working standard solutions.* Prepare the daily standard curve by diluting the stock solution described in paragraph (b)(1)(iii) of this section in sterile distilled water to the following final concentrations: 23.9, 26.8, 30.0, 33.6 and 37.6 micrograms per milliliter. Add 1 milliliter of each final dilution to each of three tubes having an outside dimension of 16 millimeters x 125 millimeters. Add 9 milliliters of inoculated broth described in paragraph (b)(1)(x)(*a*) of this section, to each tube and immediately place in a 37° C. water bath for 3 to 4 hours. After incubation, remove the tubes and add 0.5 milliliter of 12 percent formaldehyde to each tube.

(*c*) *Preparation of sample.* Dissolve the sample under test in sterile distilled water to prepare a convenient stock solution. Further dilute this stock solution in sterile distilled water to a final concentration of 30 micrograms per milliliter. Add 1 milliliter of this final concentration to each of three 16 millimeters x 125 millimeters tubes (outside dimensions). Add 9 milliliters of the inoculated broth described in paragraph (b)(1)(x)(*a*) of this section to each tube and immediately place in a 37° C. water bath for 3 to 4 hours. (The sample tubes are placed in the water bath at the same time as the standard tubes.) After incubation, remove all tubes (sample and standard) and add 0.5 milliliter of 12 percent formaldehyde to each tube. Read the absorbance values of all tubes in a suitable photo-electric colorimeter, using a wavelength of 530 mμ. Set the instrument at zero absorbance, using clear, uninoculated broth prepared as described in § 440.80a(b)(1)(ii)(*c*) of this chapter.

(*d*) *Estimation of potency.* Plot the average values for each concentration of the standard on 1-cycle semilogarithmic paper with absorbance on the arithmetic scale and concentrations on

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

the logarithmic scale. Construct the best straight line through the points, either by inspection or by means of the equations described in § 436.515(a)(1)(v) of this chapter. Average the absorbance values for the sample and read the streptomycin concentration from the standard curve. Multiply the concentration by appropriate dilution factors to obtain the streptomycin content of the sample tested.

(xi) *Potency.* The potency of streptomycin is satisfactory, when assayed by the methods described in this section, if the immediate containers contain 90 percent of the number of grams they are represented to contain.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Toxicity.* Proceed as directed in § 440.80a(b)(4) of this chapter, using as a test dose 0.5 milliliter of a solution containing 2 mg./ml.

(4) *Pyrogens.* Proceed as directed § 440.80a(b)(3) of this chapter, using as a test dose 1.0 milliliter per kilogram of a solution containing 10 mg./ml.

(5) *Histamine.* Use a healthy adult cat as the test animal. Determine weight and place under general anesthesia by intraperitoneal injection of any suitable medium- or long-acting anesthetic which does not unduly affect the level or stability of the blood pressure. Surgically expose the common carotid artery, separating it completely from all surrounding structures, including the vagus nerve, by blunt dissection. Cannulate for direct blood pressure measurement. Surgically expose the femoral vein. Start the recording kymograph and inspect the tracings for amplitude of excursion and relative stability of pressure. Determine the sensitivity of the animal by injecting into the femoral vein standard solutions of histamine made to contain the equivalent of 1.0 microgram of histamine base per milliliter. Make injections at not less than 5-minute intervals using doses of 0.05, 0.1, and 0.15 microgram of histamine base per kilogram. Repeat these injections, disregarding the first series of readings, until the drop given by equivalent doses of histamine is relatively uniform. The fall in blood pressure given by 0.1 mcg./kg. of histamine base (not less than 20 millimeters of mercury) is subsequently employed as the standard in testing samples. The histamine standard is supplied on request. Inject 3 mg./kg. of the sample of streptomycin per milliliter maintaining the 5-minute injection schedule. If a significant drop is encountered the dose is repeated after the animal has been retested with the standard histamine. The animal may be used as long as it remains reasonably stable and responsive to histamine. The product is satisfactory if the fall in blood pressure obtained with 3 milligrams of streptomycin per kilogram of body weight is no greater than the fall obtained with 0.1 microgram of histamine base per kilogram of body weight. (Dogs may be substituted for cats in this test provided the ratio of the doses of streptomycin and histamine employed is the same.)

(6) *Moisture, pH, and identity*—(i) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) of this chapter.

(ii) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using a solution with a concentration of 0.2 gm./ml.

(iii) *Identity.* Using distilled water, dilute the sample to be tested to a concentration of approximately 1,000 micrograms per milliliter. To 5.0 milliliters of this solution, add 2.0 milliliters of 1*N* NaOH and heat in a boiling water bath for 10 minutes. Cool in ice water for 3 minutes and then acidify the solution by adding 2.0 milliliters of 1.2*N* HCl. Add 5.0 milliliters of 0.25 percent ferric chloride reagent, prepared as directed in § 444.10a(b)(2)(i). A violet color appears.

§ 444.72a Sterile streptonicozid sulfate.

(a) *Requirements for certification.*—(1) *Standards of identity, strength, quality, and purity.* Streptonicozid sulfate is the crystalline sulfate compound of streptomycin and isonicotinic acid hydrazide. It is so purified and dried that:

(i) It contains not less than the equivalent of 583 micrograms of streptomycin per milligram and its isonicotinic acid hydrazide equivalent content is not less than 13.75 percent.

(ii) It is sterile.

(iii) It is nontoxic.

(iv) It is nonpyrogenic.

(v) It contains no histamine nor histamine-like substance.

(vi) Its moisture content is not more than 5 percent.

(vii) Its pH in an aqueous solution containing the equivalent of 0.2 gram of streptomycin per milliliter is not less than 4.5 and not more than 7.5.

(viii) Its absorptivity (1%, 1 cm.) is not less than 150 at 260 millimicrons.

The streptomycin used conforms to the requirements of § 444.70a(a)(1). The isonicotinic acid hydrazide used has a purity of not less than 98 percent and it has a melting point of not less than 169° C. and not more than 172° C.

(2) *Packaging.* It shall be packaged in accordance with the requirements of § 444.70a(a)(2), except that each immediate container shall contain the equivalent of not less than 1.0 gram of streptomycin.

(3) *Labeling.* In addition to the labeling requirements of § 444.70a(a)(3)(i) each package shall bear on the outside wrapper or container and the immediate container the number of grams of equivalent streptomycin activity, the equivalent number of grams of isonicotinic acid hydrazide, and the number of grams of streptonicozid sulfate in the immediate container.

(4) *Request for certification, check tests and assays; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in the batch, the number of grams of streptomycin, isonicotinic acid hydrazide, and streptonicozid sulfate in each package and (unless it was previously submitted) the date on which the latest assay of the drug comprising such batch was completed. Such request shall be accompanied or followed by the results of tests and assays made by him on the batch for potency, sterility, toxicity, pyrogens, histamine content, moisture, pH, crystallinity, and absorptivity. If such batch or any part thereof is to be packaged with a solvent, such request shall also be accompanied by a statement that such solvent conforms to the requirements prescribed therefor by this section.

(ii) If such batch is packaged for dispensing, such person shall submit in connection with his request an accurately representative sample of the batch, consisting of the following:

(*a*) For all tests except sterility: One immediate container for each 5,000 immediate containers in the batch, but in no case less than six immediate containers.

Such samples shall be collected by taking single immediate containers at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal:

(*b*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(iii) If such batch is packaged for repacking or for use in the manufacture of another drug, such person shall submit with his request an accurately representative sample of the batch, consisting of the following:

(*a*) For all tests except sterility: six packages, each containing the equivalent of approximately 1 gram of streptomycin activity.

(*b*) For sterility testing: 20 packages, each containing approximately 0.3 gram. Each such package shall be taken from a different part of such batch, and each shall be packaged in accordance with the requirements of paragraph (a)(2) of this section.

(iv) In connection with contemplated requests for certification of repackaged batches or batches of another drug in the manufacture of which it is to be used, the manufacturer of the batch which is to be so repacked or used may request the Commissioner to make check tests and assays on a sample of such batch, taken as prescribed by paragraph (a)(4)(iii) of this section. From the information required by paragraph (a)(4)(i) of this section may be omitted tests and results of assays not required for the batch when used in such other drug. The Commissioner shall report to such manufacturer results of such check tests and assays as are so requested.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Streptomycin content.* Dilute the sample with hydrochloric acid solution pH 1.5 to 1,000 micrograms per milliliter (estimated streptomycin activity). Heat at 50° C. for 30 minutes, cool rapidly and proceed as directed in

§ 444.70a(b)(1). The streptomycin potency of streptonicozid sulfate is satisfactory if the immediate containers contain 90 percent of the streptomycin activity they are represented to contain.

(ii) *Isonicotinic acid hydrazide content*—(*a*) *Reagents.* (*1*) 0.1*N* $KBrO_3$-KBr solution: Dissolve 3 grams of $KBrO_3$ and 15 grams of KBr in sufficient water to make 1,000 milliliters.

(*2*) Concentrated hydrochloric acid.

(*3*) 20-percent potassium iodide solution.

(*4*) 0.05*N* $Na_2S_2O_3$ (accurately standardized against KIO_3).

(*b*) *Preparation of sample.* Place an accurately weighed sample of approximately 250 milligrams or an aliquot of a solution containing 250 milligrams of streptonicozid sulate into a 250-milliliter iodine flask. Add sufficient water to give a volume of 25 milliliters.

(*c*) *Blank.* Add 25 milliliters of distilled water in a 250-milliliter iodine flask.

(*d*) *Procedure.* To each iodine flask containing the sample and blank, add 25 milliliters of 0.1*N* $KBrO_3$-KBr solution and 5 milliliters of concentrated HCl. Stopper the flasks, and place distilled water in the wells around the stopper. Allow the flasks to stand for 15 minutes at room temperature, and then add 5 milliliters of 20-percent potassium iodide solution to each flask. Titrate the liberated iodine with 0.05*N* $Na_2S_2O_3$, using starch as an indicator.

(*e*) *Calculations.*

$$\text{Percent isonicotine acid hydrazide} = \frac{(B-S)\times N\times 3429}{W}$$

where:

B = milliliters of $Na_2S_2O_3$ required for the blank.
S = milliliters of $Na_2S_2O_3$ required for the sample.
N = normality of the $Na_2S_2O_3$.
W = milligrams of sample.

(2) *Absorptivity.* Accurately weigh approximately 100 milligrams of the sample; dissolve in distilled water, and make to 100 milliliters. Dilute a 5-milliliter aliquot of this solution to 100 milliliters, allow to stand at room temperature for 15 minutes, and, using a suitable spectrophotometer, determine the absorbance of the solution in a 1-centimeter cell at 260 mμ compared with distilled water as a blank.

$$\text{Absorptivity (1\%, 1 cm.)} = \frac{\text{Absorbance at 260 m}\mu\times 20{,}000}{\text{Milligrams of sample}}.$$

(3) *Toxicity.* Proceed as directed under § 440.80a(b)(4) of this chapter, using as a test dose 0.5 milliliter of a solution containing 1,000 micrograms of streptomycin activity per milliliter.

(4) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(5) *Pyrogens, histamine, moisture, pH, and crystallinity.* Proceed as directed under §§ 444.70a(b) (4), (5), and (6), and 440.80a(b)(5)(iii) of this chapter.

Subpart B—Oral Dosage Forms

§ 444.130 Kanamycin sulfate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Kanamycin sulfate capsules are composed of crystalline kanamycin sulfate, with or without one or more suitable and harmless buffer substances, vegetable oils, preservatives, diluents, binders, lubricants, colorings, and flavorings, enclosed in gelatin capsules. Each capsule contains 500 milligrams of kanamycin. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of kanamycin that it is represented to contain. The loss on drying is not more than 4.0 percent. The crystalline kanamycin sulfate used conforms to the standards prescribed by § 444.30(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The kanamycin sulfate used in making the batch for potency, safety, loss on drying, pH, residue on ignition, identity, kanamycin B content, and crystallinity.

(*b*) The batch for potency and loss on drying.

(ii) Samples required:

(*a*) Kanamycin sulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: Minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar with sufficient sterile distilled water to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with sterile distilled water to the reference concentration of 10 micrograms of kanamycin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

§ 444.142 Neomycin sulfate oral dosage forms.

§ 444.142a Neomycin sulfate tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate tablets are tablets composed of neomycin sulfate with one or more suitable and harmless binders, and with or without one or more suitable and harmless fillers, buffers, lubricants, and colorings. Each tablet contains 150 milligrams, 175 milligrams, or 350 milligrams of neomycin. The moisture content is not more than 10.0 percent. Tablets shall disintegrate within 1 hour. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1) (i), (iv), (v), (vi), and (vii). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, toxicity, moisture, PH, and identity.

(*b*) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except disintegration time: Minimum 30 tablets.

(*2*) For disintegration time: Six tablets.

(*c*) In the case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 444.42a(b)(1), except prepare the sample as follows: Place a representative number of tablets into a high-speed glass blender, add a sufficient quantity of 0.1*M* potassium phosphate buffer, pH 8.0, to give a stock solution of convenient concentration. Blend 3 to 5 minutes. Further dilute in 0.1*M* potassium phosphate buffer, pH 8.0, to the proper prescribed reference concentration. Its neomycin content is satisfactory if it contains not less than 90 percent and not more than 125 percent of the number of milligrams of neomycin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 440.180a(b)(3) of this chapter.

§ 444.142b Neomycin sulfate oral solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate oral solution is neomycin sulfate with or without one or more suitable and harmless flavorings, colorings, and preservatives in an aqueous vehicle. Each milliliter contains 17.5 milligrams of neomycin. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of neomycin that it is represented to contain. Its pH is not less than 5.0 and not more than 7.5. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1) (i), (iv), (v), (vi), and (vii).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, toxicity, moisture, pH, and identity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 6 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, except prepare the sample as follows: Remove an accurately measured representative portion with a suitable syringe, and dilute with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted sample.

§ 444.150 Paromomycin sulfate oral dosage forms.

§ 444.150a Paromomycin sulfate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Paromomycin sulfate capsules are paromomycin sulfate enclosed in a suitable and harmless gelatin capsule. Each capsule contains 250 milligrams of paromomycin. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of paromomycin that it is represented to contain. The loss on drying is not more than 7.0 percent. The paromomycin sulfate used conforms to the standards prescribed therefor by § 444.50(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The paromomycin sulfate used in making the batch for potency, safety, loss on drying, pH, specific rotation, and residue on ignition.

(*b*) The batch for potency and loss on drying.

(ii) Samples required:

(*a*) The paromomycin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Blend a representative number of capsules for 3 to 5 minutes in a high-speed glass blender with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute the stock solution with solution 3 to the reference concentration of 1.0 microgram of paromomycin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

§ 444.150b Paromomycin sulfate sirup.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Paromomycin sulfate sirup contains the equivalent of 25 milligrams of paromomycin per milliliter. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of paromomycin that it is represented to contain. It may contain one or more suitable and harmless solvents, flavorings, colorings, preservatives, and buffers in water. Its pH is not less than 7.5 and not more than 8.5. The paromomycin sulfate used conforms to the requirements of § 444.50(a)(1) (i), (ii), (iv), (v), and (vi).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays for:

(*a*) The paromomycin sulfate used in making the batch for potency, safety, pH, specific rotation, and residue on ignition.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The paromomycin sulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed § 436.105 of this chapter, preparing the sample for assay as follows: Remove an appropriate aliquot of the sirup and transfer to an appropriate-sized volumetric flask. Dilute to volume with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and mix well. Further dilute with solution 3 to the reference concentration of 1.0 microgram of paromomycin per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted sample.

§ 444.170a Streptomycin-polymyxin-bacitracin tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Streptomycin-polymyxin-bacitracin tablets are tablets composed of streptomycin, polymyxin B, and bacitracin, with or without the addition of one or more suitable and harmless buffer substances, diluents, binders, lubricants, colorings, and flavorings. The potency of each tablet is not less than 250 milligrams of streptomycin, 200,000 units of polymyxin B, and 5,000 units of bacitracin. Its moisture content is not more than 3 percent. Tablets not exceeding 15 millimeters in diameter, or not intended only for preparing solutions, shall disintegrate within 1 hour. The streptomycin used conforms to the standards prescribed therefor by § 444.70a(a)(1), except § 444.70a (a)(1) (ii), (iv), and (v). The polymyxin used is produced by the growth of *Bacillus polymyxa,* has a potency of not less than 3,800 units per milligram, and is nontoxic. The bacitracin used conforms to the standards prescribed therefor by § 448.10a(a)(1) of this chapter, except § 448.10a(a)(1) (ii), (iv), and (viii). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* Unless each tablet is enclosed in a foil or plastic film and such enclosure is a tight container as defined by the U.S.P., except the provision that it shall be capable of tight reclosure, the immediate container shall be a tight container as so defined. The immediate container may also contain a desiccant separated from the tablets by a plug of cotton or other like material. The composition of the immediate container, or of the foil or film enclosure, shall be such as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106 (b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on the outside wrapper or container and the immediate container the statement, "Expiration date _____", the blank being filled in with the date that is 18 months after the month during which the batch was certified.

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch of streptomycin-polymyxin-bactracin tablets shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless they were previously submitted) the dates on which the latest assays of the streptomycin, polymyxin, and bacitracin used in making such batch were completed, the potency of each tablet, the quantity of each ingredient used in making the batch, the date on which the latest assay of the drug comprising such batch was completed, and a statement that each ingredient used in making the batch conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided in paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request results of the tests and assays listed after each of the following, made by him on an accurately representative sample of;

(*a*) The batch: Average potency per tablet, average moisture, and if required

by paragraph (a) (1) of this section, disintegration time.

(b) The streptomycin used in making the batch; potency, toxicity, moisture, and pH.

(c) The polymyxin used in making the batch; potency and toxicity.

(d) The bacitracin used in making the batch; potency, toxicity, moisture, and pH.

(iii) Except as otherwise provided by paragraph (a) (4) (iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(a) The batch:

(1) For potency and moisture: One tablet for each 5,000 tablets in the batch, but in no case less than 30 tablets, collected by taking single tablets throughout the entire time of tableting so that the quantities tableted during the intervals are approximately equal.

(2) For disintegration time: 6 tablets.

(b) The streptomycin used in making the batch; five packages containing approximately equal portions of not less than 0.5 gram each, packaged in accordance with the requirements of § 444.70a (a) (2).

(c) The polymyxin used in making the batch; five packages, each containing approximately equal portions of not less than 0.5 gram.

(d) The bacitracin used in making the batch; six packages, each containing approximately equal portions of not less than 0.5 gram, packaged in accordance with the requirements of § 448.10a(a) (2) of this chapter.

(e) In case of an initial request for certification, each other ingredient used in making the batch; one package of each, containing approximately 5 grams.

(iv) No result referred to in paragraph (a) (4) (ii) (b), (c), and (d) of this section, and no sample referred to in paragraph (a) (4) (iii) (b), (c), and (d) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay*—(1) *Tablets*—(i) *Potency*—(a) *Streptomycin content.* Using 12 tablets, proceed as directed in § 141b.109(a) (1) of this chapter. Its content of streptomycin is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(b) *Polymyxin content.* Using 12 additional tablets, proceed as directed in paragraph (b) (2) of this section. Its content of polymyxin is satisfactory if it contains not less than 85 percent of the number of units that is is represented to contain.

(c) *Bacitracin content.* Proceed as directed in § 448.110a(b) (1) of this chapter, except use the test organism and prepare the standard curve as directed in § 436.505(a) (2) (ii) of this chapter. Its content of bacitracin is satisfactory if it contains not less than 85 percent of the number of units that it is represented to contain.

(ii) *Moisture.* Proceed as directed in § 440.80a(b) (5) (i) of this chapter.

(iii) *Disintegration time.* Proceed as directed in § 440.180a(b) (3) of this chapter.

(2) *Polymyxin used in making the tablets*—(i) *Potency*—(a) *Cylinders (cups).* Use cylinder described under § 440.80a(b) (1) (i) of this chapter.

(b) *Culture medium.* Using ingredients that conform to the standards prescribed, if any, by the U. S. P. or N. F., make nutrient agar for the seed and base layers:

(1) *Base layer.*

Pancreatic digest of casein	17.0 gm.
Papaic digest of soybean	3.0 gm.
Sodium chloride	5.0 gm.
Dipotassium phosphate	2.5 gm.
Dextrose	2.5 gm.
Agar	20.0 gm.
Distilled water, q. s.	1,000.0 ml.
Final pH	7.8.

(2) *Seed layer.* For seed layer make the following changes in the medium described in paragraph (b) (2) (i) (a) (1) of this section.

Agar	12.0 gm.
Tween 80 (add Tween 80 after boiling)	10.0 gm.
Final pH	7.3.

In lieu of preparing the medium from the individual ingredients as specified, it may be prepared from a dehydrated mixture which, when reconstituted with distilled water, has the same composition as such medium. Minor modifications of the specified individual ingredients are permissible if the resulting medium possesses growth-promoting properties at least equal to the medium described.

(c) *Working standard.* Weigh out a sufficient quantity of the working standard (obtained from the U.S.P. Reference Standards Committee, 46 Park Avenue New York 16, NY), dried as described in § 440.80a(b) (5) (i) of this chapter, and dissolve in 2.0 milliliters of distilled water, then add sufficient 10 percent potassium phosphate buffer, pH 6.0, to make a 1,000 units per milliliter stock solution. This solution may be used for 2 weeks if kept in refrigerator.

(d) *Standard curve.* Prepare daily a standard curve as directed in § 436.105 (b), (c), and (d) of this chapter, with the following exceptions: Using a solution of the polymyxin working standard in 10 percent potassium phosphate buffer, pH 6.0, prepare volumetrically the following concentrations: 6.4, 8.0, 10.0, 12.5, and 15.6 units per milliliter in 10 percent potassium phosphate buffer, pH 6.0. The 10 units per milliliter concentration is used as the reference point.

(e) *Preparation of test organism.* The test organism is *Brucella bronchiseptica* (American Type Culture Collection 4617)[1] which is maintained on agar described under paragraph (b) (2) (i) (b) (1) of this section. Inoculate a Roux bottle containing this agar, paragraph (b) (2) (i) (b) (1), from a stock slant and incubate 24 hours at 32° C.–35° C. Wash the growth into sterile distilled water and standardize the resulting organism suspension to 50-percent light transmission using a filter having a wave length of 6,500 A. Run test plates, and use in the assay the amount of inoculum per 10 milliliters of seed agar which gives (a) 15.0-millimeter zones of inhibition against the test organism with the 100 units per milliliter solution of polymyxin. Make a new suspension every two weeks.

(f) *Preparation of plates.* Using the agar described in paragraph (b) (2) (i) (b) of this section, prepare the plates as described in § 444.70a(b) (1) (vii), except allow the agar for the secondary layer to cool to 48° C.—50° C. before adding the inoculum. It is not necessary to refrigerate the plates.

(g) *Assay.* Dissolve in sufficient sterile distilled water to give a concentration of 30,000 units (estimated) per milliliter. Add sufficient 10 percent potassium phosphate buffer, pH 6.0 to make an appropriate stock solution. Dilute further in sterile 10 percent potassium phosphate buffer, pH 6.0, to a final concentration of 10 units (estimated) per milliliter, and proceed as directed in § 444.70a(b) (1) (viii) and (ix).

(ii) *Toxicity.* Proceed as directed in § 440.80a(b) (4) of this chapter, using 0.5 milliliter of a solution prepared by diluting the sample to approximately 1,200 units per milliliter with physiological salt solution.

Subpart C—Injectable Dosage Forms

§ 444.220 Gentamicin sulfate injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Gentamicin sulfate injection is an aqueous solution of gentamicin sulfate and one or more suitable buffers, sequestering agents, and preservatives. Each milliliter contains gentamicin sulfate equivalent to either 10 milligrams or 40 milligrams of gentamicin. Its potency is satisfactory if is contains not less than 90 percent nor more than 125 percent of the number of milligrams of gentamicin that it is represented to contain. It is sterile. It passes the safety test. It is nonpyrogenic. Its pH is not less than 3.0 nor more than 5.5. The gentamicin sulfate used conforms to the standards prescribed therefor by § 444.20a(a) (1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The gentamicin sulfate used in making the batch for potency, loss on drying, pH, specific rotation, content of gentamicins C_1, C_{1a}, and C_2, and identity.

(b) The batch for gentamicin potency, sterility, safety, pyrogens, and pH.

(ii) Samples required:

(a) The gentamicin sulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 40 vials if each milliliter contains the equivalent of 10 milligrams of gentamicin of a minimum of 12 vials if each milliliter contains the equivalent of 40 milligrams of gentamicin.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Using 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), dilute an accurately measured representative portion of the product to the reference concentration of 0.1 microgram of gentamicin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Safety test.* Proceed as directed in § 436.33 of this chapter.

(4) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 10.0 milliliters of gentamicin per milliliter.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted solution.

§ 444.230 Kanamycin sulfate injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Kanamycin sulfate injection is an aqueous solution of kanamycin sulfate with suitable and harmless buffer substances and preservatives. It contains either 75 milligrams of kanamycin per 2.0 milliliters, or 250 milligrams of kanamycin per milliliter, or 1.0 gram of kanamycin per 3.0 milliliters. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of kanamycin that it is represented to contain. It is sterile. It passes the safety test. It is nonpyrogenic. Its pH is not less than 3.5 and not more than 5.0. The kanamycin sulfate used conforms to the standards prescribed by § 444.30a(a)(1) (i), (v), (vii), (viii), (ix), and (x).

(2) *Labeling.* In addition to the requirements prescribed by § 432.5 of this chapter, the labeling of each package shall bear a warning to the effect that older patients and patients receiving a total dose of more than 20 grams of the drug should be carefully observed for signs of eighth-nerve damage. In patients with impaired kidney function or with prerenal azotemia, the risk of severe ototoxic reaction that may result in permanent deafness is sharply increased.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The kanamycin sulfate used in making the batch for potency, residue on ignition, loss on drying, identity, crystallinity, and kanamycin B content.

(*b*) The batch for potency, sterility, safety, pyrogens, and pH.

(ii) Samples required:

(*a*) The kanamycin sulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: Minimum of 12 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place an accurately measured representative aliquot of the sample into an appropriate-sized volumetric flask and dilute to volume with sterile distilled water to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 10 micrograms of kanamycin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Safety.* Proceed as directed in § 436.33 of this chapter.

(4) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 10 milligrams of kanamycin per milliliter.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted solution.

§ 444.270 Streptomycin sulfate injectable dosage forms.

§ 444.270a Sterile streptomycin sulfate, sterile streptomycin hydrochloride, sterile streptomycin phosphate, sterile streptomycin trihydrochloride calcium chloride (streptomycin calcium chloride complex).

The requirements for certification and the tests and methods of assay for sterile streptomycin sulfate, sterile streptomycin hydrochloride, sterile streptomycin phosphate, and sterile streptomycin trihydrochloride calcium chloride streptomycin calcium chloride complex), packaged for dispensing, are described in § 444.70a.

§ 444.270b Streptomycin sulfate injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Streptomycin sulfate injections is an aqueous solution of streptomycin sulfate. Such solution conforms to all standards prescribed by § 444.70a(a) for streptomycin sulfate, except:

(i) The limitation on moisture content does not apply.

(ii) Its potency is not less than 250 milligrams per milliliter and not more than 500 milligrams per milliliter.

(iii) The histamine test may be omitted if it has been performed on streptomycin sulfate, used in preparing the solution.

(iv) It contains one or more suitable and harmless preservatives.

(v) Its pH is not less than 5.0 and not more than 8.0.

(vi) It may contain one or more suitble and harmless buffer substances and stabilizing agents.

(2) *Packaging.* In all cases the immediate container shall be a tight container as defined by the U.S.P., shall be sterile at the time of filling and closing, shall be so sealed that the contents cannot be used without destroying the seal, and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling*—(i) *It is packaged for dispensing.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its label or labeling, as hereinafter indicated, the following:

(*a*) On the outside wrapper or container and the immediate container, the statement "Expiration date _____", the blank being filled in with the date that is 12 months after the month during which the batch was certified except that the blank may be filled in with the date that is 18 months, 24 months, 36 months, 48 months, or 60 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period such drug as prepared by him complies with the standards prescribed by paragraph (a)(1) of this section.

(*b*) On the outside wrapper or container the statement "Store in refrigerator not above 15° C. (59° F.)" or "Store below 15° C. (59° F.)" unless the person who requests certification has submitted to the Commissioner results of tests and assays showing that such drug as prepared by him complies with the standards prescribed by paragraph (a)(1) of this section after having been stored at room temperature.

(ii) *It is packaged for manufacturing use and/or repacking.* Each package shall bear on its outside wrapper or container and the immediate container the following:

(*a*) The number of milligrams in each milliliter and the number of milliliters in the immediate container.

(*b*) The statement "Caution: Federal law prohibits dispensing without prescription".

(*c*) The statement "For manufacturing use", "For repacking", or "For manufacturing use or repacking".

(*d*) The information required by paragraph (a)(3)(i) of this section.

(4) *Request for certification, check tests and assays; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in the batch, the number of milligrams or grams dissolved in each of such packages, and the date on which the latest assay of the drug comprising such batch was completed.

(ii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request results of the tests and assays listed after each of the following, made by him on an accurately representative sample of:

(*a*) The batch; potency, sterility, toxicity, pyrogens, histamine content (except that the result of this test performed

on the streptomycin sulfate used in making the batch may be submitted instead) and pH.

(*b*) The streptomycin sulfate used in making the batch; potency on dry basis.

(iii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch, if packaged for dispensing:

(*1*) For all tests except sterility: One immediate container for each 5,000 immediate containers in such batch; but in no case less than five immediate containers.

Such samples shall be collected by taking single immediate containers at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(*2*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(*b*) The batch, if packaged for use in the manufacture of another drug:

(*1*) For all tests except steriity: Five packages.

(*2*) For sterility testing: 20 packages.

Each such package shall contain approximately 2 milliliters, taken from a different part of such batch, and each shall be packaged in accordance with the requirements of paragraph (a)(2) of this section.

(*c*) The streptomycin used in making the batch; one immediate container. Each immediate container shall contain approximately 0.5 gram or the dried drug packaged in accordance with the requirements for veterinary use of § 444.-70a(a)(2). If the streptomycin used in making the batch is a solution of the drug, the person who requests certification shall dry a sufficient quantity of such solution for potency testing on the dry basis.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch; one package of each containing approximately 5 grams.

(iv) No result referred to in paragraph (a)(4)(ii)(*b*) of this section, and no sample referred to in paragraph (a)(4)(iii)(*c*) of this section, is required if such result or sample has been previously submitted.

(v) In connection with contemplated requests for certification of repackaged batches or batches of another drug in the manufacture of which it is to be used, the manufacturer of the batch which is to be so repacked or used may request the Commissioner to make check tests and assays on a sample of such batch, taken as prescribed by paragraph (a)(4)(iii)(*b*) of this section. From the information required by paragraph (a)(4)(ii)(*a*) of this section may be omitted results of tests and assays not required for the batch when used in such other drug. The Commissioner shall report to such manufacturer results of such check tests and assays as are so requested.

(b) *Tests and methods of assay.* Proceed as directed in §§ 444.70a(b)(1), (2), (4), and (5) (the histamine test may be omitted if it is performed on the streptomycin sulfate used in preparing the injection), and § 444.70a(b)(6)(ii) and (iii), and for the toxicity test proceed as directed in § 440.80a(b)(4) of this chapter, using as a test dose 0.5 milliliter of a solution containing 1.5 milligrams per milliliter.

§ 444.270c Streptomycin sulfate for injection.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Streptomycin sulfate for injection conforms to all the requirements and is subject to all procedures prescribed by § 444.70a(a)(1) for streptomycin sulfate except that:

(i) It contains the buffer sodium citrate in a quantity not more than 4.0 percent by weight of its total solids. The sodium citrate conforms to the standards prescribed therefor by the U.S.P.

(ii) It may contain sodium bisulfite as a stabilizer in a quantity not more than 0.8 percent by weight of its total solids. The sodium bisulfite conforms to the standards prescribed therefor by the U.S.P.

(2) *Packaging.* It shall be packaged in accordance with the requirements of § 432.1 of this chapter.

(3) *Labeling—*(i) *It is packaged for dispensing.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its label or labeling, as hereinafter indicated, the following:

(*a*) On the outside wrapper or container and the immediate container, the statement "Expiration date ----------" the blank being filled in with the date that is 24 months after the month during which the batch was certified.

(*b*) On the circular or labeling within or attached to the package, the conditions under which solutions of the drug should be stored, including the statement "Sterile solutions may be stored at room temperature for 4 weeks without significant loss of potency".

(ii) *It is packaged solely for manufacturing use and/or repacking.* Each package shall bear on its outside wrapper or container and the immediate container the following:

(*a*) The potency per gram and the number of grams in the immediate container.

(*b*) The statement "Caution: Federal law prohibits dispensing without prescription".

(c) The statement "For manufacturing use", "For repacking", or "For manufacturing use or repacking".

(d) The information required by paragraph (a)(3)(i)(*a*) of this section.

(4) *Request for certification, check tests, and assays; samples.* Proceed as directed in § 444.70a(a)(4), except that a person who requests certification of a batch shall also submit with his request a statement showing the quantities of sodium citrate and sodium bisulfite used in making the batch and that such sodium citrate and sodium bisulfite conform to the requirements prescribed therefor by this section. In case of an initial request for certification, he shall submit accurately representative samples of such sodium citrate and sodium bisulfite consisting of approximately 5 grams each.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 444.70a(b)(1). Its content or streptomycin is satisfactory if the immediate containers contain not less than 90 percent and not more than 115 percent of the number of grams of streptomycin that they are represented to contain.

(2) *Sterility, toxicity, pyrogens, histamine, moisture, pH, and identity.* Proceed as directed in § 444.70a(b)(2), (3), (4), (5), and (6).

Subpart D—Ophthalmic Dosage Forms

§ 444.320 Gentamicin sulfate ophthalmic dosage forms.

§ 444.320a Gentamicin sulfate ophthalmic solution.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Gentamicin sulfate ophthalmic solution contains in each milliliter the equivalent of 3.0 milligrams of gentamicin and suitable buffers and preservatives. Its potency is satisfactory if it is not less than 90 and not more than 135 percent of the number of milligrams of gentamicin it is represented to contain. It is sterile. Its pH is not less than 6.5 nor more than 7.5. The gentamicin sulfate conforms to the standards prescribed by § 444.20(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The gentamicin sulfate used in making the batch for potency, safety, loss on drying, pH, specific rotation, content of gentamicins C_1, C_{1a}, and C_2, and identity.

(*b*) The batch for potency, sterility, and pH.

(ii) Samples required:

(*a*) The gentamicin sulfate used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of five immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the product with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 0.1 microgram of gentamicin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted sample.

§ 444.320b Gentamicin sulfate ophthalmic ointment.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Gentamicin sulfate ointment contains in each gram the equivalent of 3.0 milligrams of gentamicin with suitable preservatives in a white petrolatum base. Its potency is satisfactory if it is not less than 90 percent and not more than 135 percent of the number of milligrams of gentamicin that it is represented to contain. It is sterile. Its moisture content is not more than 1.0 percent. It passes the test for particulate contamination. The gentamicin sulfate used conforms to the standards prescribed therefor by § 444.20a (a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The gentamicin sulfate used in making the batch for potency, safety, loss on drying, pH, specific rotation, content of gentamicins C_1, C_{1a}, and C_2, and identity.

(*b*) The batch for gentamicin potency, sterility, moisture, and particulate contamination.

(ii) Samples required:

(*a*) The gentamicin sulfate used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 15 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 436.105 of this chapter, except prepare the sample as follows: Place an accurately weighed representative portion of the ointment into a separatory funnel containing 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction with new portions of solution 3. Repeat any additional times necessary to insure complete extraction of the antibiotic. Combine the extractives and adjust to an appropriate volume to give a stock solution of convenient concentration. Further dilute with solution 3 to the reference concentration of 0.1 microgram of gentamicin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(3) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *Particulate contamination.* Proceed as directed in § 436.206 of this chapter.

§ 444.342 Neomycin sulfate ophthalmic dosage forms.

§ 444.342a Neomycin sulfate-_____ ophthalmic suspension; neomycin sulfate-_____ ophthalmic solution (the blanks being filled in with the established name(s) of the other active ingredient(s) present in accordance with paragraph (a)(1) of this section).

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* The drug is a suspension or a solution containing, in each milliliter, 3.5 milligrams of neomycin and the following other active ingredients in a suitable and harmless vehicle:

(i) 15 milligrams of cortisone acetate; or

(ii) 5 milligrams or 25 milligrams of hydrocortisone acetate; or

(iii) 1 milligram or 2 milligrams of prednisolone; or

(iv) 1 milligram of sodium dexamethasone phosphate.

It may also contain one or more suitable and harmless buffers, dispersants, and preservatives. It is sterile. Its pH is not less than 6.0 and not more than 8.0. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1) (i), (iv), (vi), and (vii). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, toxicity, pH, and identity.

(*b*) The batch for potency, sterility, and pH.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 containers, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 5 immediate containers.

(*2*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 444.442a (b)(1). Its neomycin content is satisfactory if it contains not less than 90 percent and not more than 130 percent of the number of milligrams of neomycin that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except if the steroid prevents solubilization, use 0.25 milliliter of sample in lieu of 1 milliliter and proceed as directed in paragraph (e)(2) of that section.

(3) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using the undiluted sample.

§ 444.342b Neomycin sulfate-polymyxin B sulfate-gramicidin ophthalmic solution.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate-polymyxin B sulfate-gramicidin ophthalmic solution is a solution containing, in each milliliter, 1.75 milligrams of neomycin, 5,000 units of polymyxin B, and 0.025 milligram of gramicidin, and with one or more suitable and harmless buffers, dispersants, and preservatives in a suitable and harmless isotonic aqueous vehicle. It is sterile. Its pH is not less than 4.7 and not more than 6.0. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1)(i), (iv), (vi), and (vii). The polymyxin B sulfate used conforms to the standards prescribed by § 448.30a(a)(1)(i), (iv), (vi), (vii), and (ix) of this chapter. The gramicidin used conforms to the standards prescribed by § 448.25(a)(1)(i), (ii), (iv), (v), and (vi) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, toxicity, pH, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, toxicity, pH, residue on ignition, and identity.

(*c*) The gramicidin used in making the batch for potency, toxicity, residue on ignition, melting point, crystallinity, and identity.

(*d*) The batch for neomycin content, polymyxin content, gramicidin content, sterility, and pH.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The gramicidin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*d*) The batch:

(*1*) For all tests except sterility: A minimum of 7 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regluar intervals throughout each filling operation, except that if the product is sterilized after filling, a representative sample consisting of 10 immediate containers from each sterilizer load. If only 1 sterilizer load is involved, the sample shall consist of 20 immediate containers.

(e) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Neomycin content.* Proceed as directed in § 444.42a(b)(1), except prepare the sample as follows: Remove an accurately measured portion and dilute with 0.1*M* potassium phosphate buffer, pH 8.0, to the proper prescribed reference concentration. The neomycin content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of neomycin that it is represented to contain.

(ii) *Polymyxin content.* Remove an accurately measured portion and dilute with 10 percent potassium phosphate buffer, pH 6.0, to a reference concentration of 10 units of polymyxin per milliliter. Proceed as directed in § 448.30a(b)(1) of this chapter, except add to each concentration of the polymyxin standard curve a quantity of neomycin to yield the same concentration of neomycin as that present when the sample is diluted to contain 10 units of polymyxin per milliliter. If thimerosal is an ingredient adjust the seed layer medium to contain 300 milligrams of thioglycollic acid per liter. The polymyxin content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of polymyxin that it is represented to contain.

(iii) *Gramicidin content.* Proceed as directed in § 448.25(b)(1) of this chapter, except to prepare the sample for assay remove a representative sample with a suitable syringe, place into an appropriate volumetric flask, and dilute with 95 percent alcohol to give a stock solution of convenient concentration. Make proper estimated dilutions in 95 percent alcohol to the reference concentration. The gramicidin content is satisfactory if it contains not less than 90 percent and not more than 130 percent of the number of milligrams of gramicidin that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using the undiluted sample.

§ 444.342c Neomycin sulfate-gramicidin-_____ ophthalmic solution; neomycin sulfate-gramicidin-_____ ophthalmic suspension (the blanks being filled in with the established name(s) of the other ingredient(s) present in accordance with paragraph (a)(1) of this section).

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* The drug is a solution or suspension in a suitable and harmless aqueous vehicle containing, in each milliliter, the following:

(i) 2.5 milligrams of neomycin, 0.025 milligram of gramicidin, and 1 milligram of fluorocortisone acetate; or

(ii) 2.5 milligrams of neomycin, 0.025 milligram of gramicidin, and 1.14 milligrams of fluorocortisone hemisuccinate. It may also contain suitable and harmless buffers, dispersants, irrigants, and preservatives. It is sterile. Its pH is not less than 5.0 nor more than 7.5. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1)(i), (iv), (vi), and (vii). The gramicidin used conforms to the standards prescribed by § 448.25(a)(1)(i), (ii), iv), and (vi) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, toxicity, pH, and identity.

(*b*) The gramicidin used in making the batch for potency, toxicity, crystallinity, residue on ignition, melting point, and identity.

(*c*) The batch for neomycin content, gramicidin content, sterility, and pH.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The gramicidin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*c*) The batch:

(*1*) For all tests except sterility: A minimum of 6 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Neomycin content.* Proceed as directed in § 444.342b(b)(1)(i). The neomycin content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of neomycin that it is represented to contain.

(ii) *Gramicidin content.* Proceed as directed in § 444.342b(b)(1)(iii). The content of gramicidin is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of gramicidin that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except use 0.25 milliliter of sample in lieu of 1.0 milliliter.

(3) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using the undiluted sample.

§ 444.342d Neomycin sulfate-polymyxin B sulfate-_____ ophthalmic suspension (the blank being filled in with the established name(s) of the other active ingredient(s) present in accordance with paragraph (a)(1) of this section).

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* The drug is a suspension in a suitable and harmless aqueous vehicle containing, in each milliliter, neomycin sulfate, polymyxin B sulfate, and other active ingredients in the following amounts:

(i) 3.5 milligrams of neomycin, 16,250 units of polymyxin B, and either 5 milligrams or 15 milligrams of hydrocortisone acetate; or

(ii) 5 milligrams of neomycin, 15,000 units of polymyxin B, and 2.5 milligrams of hydrocortisone.

It may contain one or more suitable and harmless irrigants, dispersants, buffers, and preservatives. It is sterile. Its pH is not less than 5.0 and not more than 7.0. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1)(i), (iv), (vi), and (vii). The polymyxin B sulfate used conforms to the standards prescribed by § 448.30a(a)(1)(i), (iv), (vi), (vii), and (ix) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, toxicity, pH, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, toxicity, pH, residue on ignition, and identity.

(*c*) The batch for neomycin content, polymyxin content, sterility, and pH.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containng approximately 300 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The batch for:

(*1*) All tests except sterility: A minimum of 6 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Neomycin content.* Proceed as directed in § 444.42a(b)(1) except prepare the sample as follows: Remove an accurately measured representative portion of the sample with a suitable syringe, place into an appropriate volumetric flask to yield a convenient stock solution. Dilute to volume with 0.1*M* potassium phosphate buffer, pH 8.0. Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0, to the proper prescribed reference concentration. Its content of neomycin is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milli-

grams of neomycin that it is represented to contain.

(ii) *Polymyxin content.* Remove an accurately measured representative portion with a suitable syringe, dilute to a convenient concentration with 10 percent potassium phosphate buffer, pH 6.0. Further dilute to a concentration of 10 units of polymyxin per milliliter with 10 percent potassium phosphate buffer, pH 6.0, and proceed as directed in § 448.30a(b)(1) of this chapter, except add to each concentration of the polymyxin standard curve a quantity of neomycin to yield the same concentration of neomycin as that present when the sample is diluted to contain 10 units of polymyxin per milliliter. Its content of polymyxin is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of units of polymyxin that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except if the steroid prevents solubilization, use 0.25 milliliter of sample in lieu of 1 milliliter and proceed as directed in paragraph (e)(2) of that section.

(3) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using the undiluted sample.

NOTE: The effective date for paragraph (a)(1) was postponed May 23, 1973. See 38 FR 13559.

§ 444.342e Neomycin sulfate ointment; neomycin sulfate- _____ ointment (the blank being filled in with the established name(s) of certain other active ingredient(s)).

The requirements for certification and the tests and methods of assay for neomycin sulfate ointment and for neomycin sulfate- _____ ointment are described in § 444.542a.

§ 444.342f Neomycin sulfate-gramicidin topical ointment; neomycin sulfate-gramicidin-triamcinolone acetonide ointment; neomycin sulfate-gramicidin-fludrocortisone acetate ointment.

The requirements for certification and the tests and methods of assay for neomycin sulfate-gramicidin topical ointment; neomycin sulfate-gramicidin-triamcinolone acetonide ointment; neomycin sulfate-gramicidin-fludrocortisone acetate ointment are described in § 444.542f.

§ 444.342g Neomycin sulfate-hydrocortisone acetate eye-ear drops; neomycin sulfate-prednisolone acetate eye-ear drops.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate-hydrocortisone acetate eye-ear drops is an aqueous suspension containing, in each milliliter, 3.5 milligrams of neomycin and 5 milligrams or 15 milligrams of hydrocortisone acetate. Neomycin sulfate-prednisolone acetate eye-ear drops is an aqueous suspension containing, in each milliliter, 3.5 milligrams of neomycin and 2.5 milligrams of prednisolone acetate. The vehicle contains one or more suitable and harmless buffers, preservatives, and dispersants. It is sterile. Its pH is not less than 5.5 and not more than 7.5. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1) (i), (iv), (v), (vi), and (vii). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Requests for certification.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, toxicity, moisture, pH, and identity.

(*b*) The batch for potency, sterility, and pH.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 5 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 444.42a(b)(1), except prepare the sample for assay as follows: Remove 1.0 milliliter with a suitable syringe, place into an appropriate-sized volumetric flask and dilute to volume with 0.1*M* potassium phosphate buffer, pH 8.0, to give a stock solution of convenient concentration. Make proper estimated dilutions to the prescribed reference concentration with 0.1*M* potassium phosphate buffer, pH 8.0. The content of neomycin is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of neomycin that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except if the steroid prevents solubilization, use 0.25 milliliter in lieu of 1 milliliter and proceed as directed in paragraph (e)(2) of that section.

(3) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using the undiluted sample.

§ 444.342h Neomycin sulfate-polymyxin B sulfate ophthalmic ointment.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate-polymyxin B sulfate ophthalmic ointment contains in each gram neomycin sulfate equivalent to 3.5 milligrams of neomycin and polymyxin B sulfate equivalent to 6,000 units of polymyxin B with suitable preservatives in a suitable and harmless ointment base. Its neomycin sulfate content is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of neomycin that it is represented to contain. Its polymyxin B sulfate content is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of units of polymyxin B that it is represented to contain. It is sterile. Its moisture content is not more than 0.5 percent. It passes the test for metal particles. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1) except sterility and pyrogens. The polymyxin B sulfate used conforms to the standards prescribed by § 448.30a(a)(1) of this chapter except sterility, pyrogens, and heavy metals.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, safety, loss on drying, pH, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, safety, loss on drying, pH, residue on ignition, and identity.

(*c*) The batch for neomycin content, polymyxin B content, sterility, moisture, and metal particles.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The batch:

(*1*) For all tests except sterility: A minimum of 16 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay—*(1) *Potency—*(i) *Neomycin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of solution 3. Combine the buffer extractives in a suitable volumetric flask and dilute to volume with solution 3. Remove an aliquot and further dilute with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(ii) *Polymyxin B content.* Proceed as directed in § 436.105 of this chapter, except add to each concentration of the polymyxin B standard response line a quantity of neomycin to yield the same

concentration of neomycin as that present when the sample is diluted to contain 10 units of polymyxin B per milliliter. Prepare the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 10 percent potassium phosphate buffer, pH 6.0 (solution 6), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of solution 6. Combine the buffer extractives in a suitable volumetric flask and dilute to volume with solution 6. Remove an aliquot and further dilute with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(3) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *Metal particles.* Proceed as directed in § 436.206 of this chapter.

§ 444.342i Neomycin sulfate-polymyxin B sulfate ophthalmic solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate-polymyxin B sulfate ophthalmic solution contains in each milliliter neomycin sulfate equivalent to 3.5 milligrams of neomycin and polymyxin B sulfate equivalent to 16,250 units of polymyxin B in a suitable and harmless aqueous vehicle. It may contain one or more suitable and harmless irrigants, dispersants, buffers, and preservatives. Its neomycin sulfate content is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of neomycin that it is represented to contain. Its polymyxin B sulfate content is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of units of polymyxin B that it is represented to contain. It is sterile. Its pH is not less than 5.0 and not more than 7.0. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1) except sterility and pyrogens. The polymyxin B sulfate used conforms to the standards prescribed by § 448.30a(a)(1) of this chapter except sterility, pyrogens, and heavy metals.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, safety, moisture, pH, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, safety, moisture, pH, residue on ignition, and identity.

(*c*) The batch for neomycin content, polymyxin B content, sterility, and pH.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The batch:

(*1*) For all tests except sterility: A minimum of 6 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Neomycin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately measured representative portion of the sample into an appropriate-sized volumetric flask with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Remove an aliquot and further dilute with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(ii) *Polymyxin B content.* Proceed as directed in § 436.105 of this chapter, except add to each concentration of the polymyxin B standard response line a quantity of neomycin to yield the same concentration of neomycin as that present when the sample is diluted to contain 10 units of polymyxin B per milliliter. Prepare the sample for assay as follows: Place an accurately measured representative portion of the sample into an appropriate-sized volumetric flask with sufficient 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to give a stock solution of convenient concentration. Remove an aliquot and further dilute with solution 6 to the reference concentration of 10.0 units of polymyxin B per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted sample.

Subpart E—Otic Dosage Forms

§ 444.442 Neomycin sulfate otic dosage forms.

§ 444.442a Neomycin sulfate-sodium propionate otic solution; neomycin sulfate-sodium propionate-prednisolone acetate otic solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate-sodium propionate otic solution is a solution containing, in each milliliter, 3.5 milligrams of neomycin and 50 milligrams of sodium propionate, in a suitable and harmless vehicle. Neomycin sulfate-sodium propionate-prednisolone acetate otic solution is a solution containing, in each milliliter, 3.5 milligrams of neomycin, 50 milligrams of sodium propionate, and 5 milligrams of prednisolone acetate, in a suitable and harmless vehicle. The preparations may also contain suitable and harmless dispersants, buffers, perfumes, and preservatives. They are sterile. Their pH is not less than 5.5 and not more than 7.0. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1) (i), (vi), and (vii). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, pH, and identity.

(*b*) The batch for potency, sterility, and pH.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of five immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(c) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 444.42a(b)(1), except dilute an accurately measured representative portion in sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a stock solution of convenient concentration. Further dilute an aliquot with 0.1*M* potassium phosphate buffer, pH 8.0, to the proper prescribed reference concentration. Its content of neomycin is satisfactory if it contains not less than 90 percent and not more than 125 percent of the number of milligrams of neomycin that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except if the steroid prevents solubilization, use 0.25 milliliter and proceed as directed in paragraph (e)(2) of that section.

(3) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using either the undiluted sample or, if the drug contains less than 10 percent water, a 1:1 dilution of the sample with distilled water.

§ 444.442b Neomycin sulfate-gramicidin-hydrocortisone-thonzylamine hydrochloride-thonzonium bromide otic solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality and purity.* Neomycin sulfate-gramicidin - hydrocortisone - thonzylamine hydrochloride-thonzonium bromide otic solution is a solution containing, in each milliliter, 0.66 milligram of neomycin, 0.05 milligram of gramicidin, 0.2 milligram of hydrocortisone, 10 milli-

grams of thonzylamine hydrochloride, and 0.5 milligram of thonzonium bromide, with one or more suitable and harmless buffers, dispersants, and preservatives, in a suitable and harmless isotonic aqueous vehicle. It is sterile. The pH is not less than 5.0 and not more than 6.0. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1) (i), (vi), and (vii). The gramicidin used conforms to the standards prescribed by § 448.25(a)(1) (i), (iv), (v), and (vi) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, pH, and identity.

(*b*) The gramicidin used in making the batch for potency, crystallinity, residue on ignition, melting point, and identity.

(*c*) The batch for neomycin content, gramicidin content, sterility, and pH.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The gramicidin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*c*) The batch:

(*1*) For all tests except sterility: A minimum of six immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Neomycin content.* Proceed as directed in § 444.342b(b)(1)(i). The neomycin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of neomycin that it is represented to contain.

(ii) *Gramicidin content.* Proceed as directed in § 444.342b(b)(1)(iii). The gramicidin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of gramicidin that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except use 0.25 milliliter of sample in lieu of 1.0 milliliter.

(3) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using the undiluted sample.

§ 444.442c Neomycin sulfate-polymyxin B sulfate-hydrocortisone otic suspension; neomycin sulfate-polymyxin B sulfate-hydrocortisone-sodium heparin otic suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate-polymyxin B sulfate-hydrocortisone otic suspension is a suspension containing, in each milliliter, 3.5 milligrams of neomycin, 10,000 units of polymyxin B, and 10 milligrams of hydrocortisone, in a suitable and harmless vehicle. Neomycin sulfate-polymyxin B sulfate-hydrocortisone-sodium heparin otic suspension is a suspension containing, in each milliliter, 20 milligrams of neomycin, 100,000 units of polymyxin B, 5 milligrams of hydrocortisone, and 2,000 units of sodium heparin, in a suitable and harmless vehicle. The preparations may also contain one or more suitable and harmless buffers, dispersants, and preservatives. They are sterile. If the vehicle is aqueous, the pH is not less than 5.5 and not more than 8.5. If the vehicle is nonaqueous, the moisture content is not more than 1.0 percent. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1) (i), (vi), and (vii). The polymyxin B sulfate used conforms to the standards prescribed by § 448.30a(a)(1), (i), (vi), (vii), and (ix) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, pH, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, pH, residue on ignition, and identity.

(*c*) The batch, for neomycin content; polymyxin content; sterility; and either pH (if aqueous vehicle), or moisture (if nonaqueous vehicle).

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing 300 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The batch:

(*1*) For all tests except sterility: A minimum of six immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Neomycin content.* Proceed as directed in § 444.442a(b)(1), except that if the vehicle is nonaqueous prepare the sample for assay as follows: Transfer an accurately measured representative portion to a high-speed glass blender. Add 1.0 milliliter of polysorbate 80 and sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a stock solution of convenient concentration. Blend 3 to 5 minutes. Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0, to the proper prescribed reference conceneration. The content of neomycin is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of neomycin that it is represented to contain.

(ii) *Polymyxin content.* Proceed as directed in § 448.30a(b)(1) of this chapter, except:

(*a*) Prepare the sample for assay by either of the following methods:

(*1*) If the vehicle is aqueous, dissolve and dilute with 10 percent potassium phosphate buffer, pH 6.0, to the reference concentration of 10 units of polymyxin per milliliter; or

(*2*) If the vehicle is nonaqueous, transfer an accurately measured representative portion to a high-speed glass blender. Add 1.0 milliliter of polysorbate 80 and sufficient 10 percent potassium phosphate buffer, pH 6.0, to give a stock solution of convenient concentration. Blend 3 to 5 minutes. Further dilute with 10 percent potassium phosphate buffer, pH 6.0, to a reference concentration of 10 units of polymyxin per milliliter; and

(*b*) Add to each concentration of the polymyxin standard curve a quantity of neomycin to yield the same concentration of neomycin as that present when the sample is diluted to contain 10 units of polymyxin per milliliter.

Its content of polymyxin is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of polymyxin that it is represented to contain.

(2) *pH.* If its vehicle is aqueous, proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using the undiluted sample.

(3) *Moisture.* If its vehicle is nonaqueous, proceed as directed in § 436.201 of this chapter.

(4) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except if the steroid prevents solubilization, use 0.25 milliliter of sample in lieu of 1 milliliter and proceed as directed in paragraph (e)(2) of that section.

§ 444.442d Neomycin sulfate ointment; neomycin sulfate- _____ ointment (the blank being filled in with the established name(s) of certain other active ingredient(s)).

The requirements for certification and the tests and methods of assay for neomycin sulfate ointment and for neomycin sulfate- _____ ointment are described in § 444.542a.

§ 444.442e Neomycin sulfate-hydrocortisone acetate eye-ear drops; neomycin sulfate-prednisolone acetate eye-ear drops.

The requirements for certification and the tests and methods of assay for neomycin sulfate-hydrocortisone acetate eye-ear drops and for neomycin sulfate prednisolone acetate eye-ear drops are described in § 444.342g.

§ 444.470a Streptomycin otic with antifungal agent; streptomycin otic with _____; dihydrostreptomycin otic with antifungal agent; dihydrostreptomycin otic with _____ (the blank being filled in with the established name of the antifungal agent).

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Streptomycin otic with antifungal agent or dihydrostreptomycin otic with antifungal agent is streptomycin or dihydrostreptomycin and one or more suitable antifungal agents and buffer substances, with or without one or more suitable and harmless preservatives, dissolved or suspended in a suitable and harmless vehicle. Its potency is not less than 2.5 milligrams per milliliter. Its pH is not less than 4.8 and not more than 6.0 The streptomycin used conforms to the standards prescribed therefor by § 444.70a(a)(1), except § 444.70a(a)(1)(ii), (iii), (iv), (v), and (vi). The dihydrostreptomycin used conforms to the standards prescribed therefor by § 444.10a(a)(1), except the standards for sterility, pyrogens, toxicity, histamine, and moisture. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* In all cases the immediate container shall be glass, so closed as to be a tight container as defined by the U.S.P., and of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package will bear on the outside wrapper or container and the immediate container the statement "Expiration date _____", the blank being filled in with the date that is 24 months after the month during which the batch was certified, except that the blank may be filled in with the date that is 36 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that such drug as prepared by him is stable for such period of time.

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless it was previously submitted) the date on which the latest assay of the streptomycin or dihydrostreptomycin used in making the batch was completed, the potency per milliliter of the batch, the date on which the latest assay of the drug comprising such batch was completed, the quantity of each ingredient used in making the batch, and a statement that each such ingredient conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request results of the tests and assays listed after each of the following, made by him on an accurately representative sample of:

(*a*) The batch: average potency per milliliter, pH.

(*b*) The streptomycin or dihydrostreptomycin used in making the batch; potency, pH, streptomycin, content if it is dihydrostreptomycin, and crystallinity if it is crystalline dihydrostretptomycin sulfate.

(iii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch: one immediate container for each 5,000 immediate containers in the batch, but in no case less than 5 immediate containers, collected by taking single immediate containers at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(*b*) The streptomycin or dihydrostreptomycin used in making the batch; 6 packages, each containing approximately equal portions of not less than 0.5 gram, packaged in accordance with the requirements of § 444.70a(a)(2).

(*c*) In case of an initial request for certification, each other ingredient used in making the batch; 1 package of each containing approximately 5 grams.

(iv) No result referred to in paragraph (a)(4)(ii)(*b*) of this section, and no sample referred to in paragraph (a)(4)(iii)(*b*) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 444.70a(b)(1), except paragraph (b)(1)(xi) thereof, and if it is dihydrostreptomycin otic with antifungal agent use the dihydrostreptomycin working standard as a standard of comparison. Its potency is satisfactory if it contains not less than 85 percent of the number of milligrams of streptomycin or dihydrostreptomycin per milliliter that it is represented to contain.

(2) *pH.* Proceed as directed in § 440.-80a(b)(5)(ii) of this chapter, using the undiluted solution or suspension.

Subpart F—Dermatologic Dosage Forms

§ 444.520 Gentamicin sulfate dermatologic dosage forms.

§ 444.520a Gentamicin sulfate ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Gentamicin sulfate ointment is gentamicin sulfate with suitable preservatives in a white petrolatum base. Each gram contains gentamicin sulfate equivalent to 1.0 milligram of gentamicin. Its potency is satisfactory if it is not less than 90 percent and not more than 135 percent of the number of milligrams of gentamicin that it is represented to contain. Its moisture content is not more than 1.0 percent. The gentamicin sulfate used conforms to the standards prescribed therefor by § 444.20(a)(1), except safety.

(2) *Packaging.* In addition to the requirements of § 432.1 of this chapter, it may be dispensed from a pressurized container wherein it is maintained in a compartment separate from the gas used to supply the pressure.

(3) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(4) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The gentamicin sulfate used in making the batch for potency, loss on drying, pH, specific rotation, content of gentamicins C_1, C_{1a}, and C_2, and identity.

(*b*) The batch for gentamicin potency and moisture.

(ii) Samples required:

(*a*) The gentamicin sulfate used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the ointment into a separatory funnel containing 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous, add 20–25 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction with new portions of the buffer and repeat any additional times necessary to insure complete extraction of the antibiotic. Combine the extractives and adjust to an appropriate volume to give a stock solution of convenient concentration. Further dilute with solution 3 to the reference concentration of 0.1 microgram of gentamicin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 444.520b Gentamicin sulfate cream.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Gentamicin sulfate cream is gentamicin sulfate with one or more

suitable emollients, dispersants, and preservatives in a suitable and harmless cream base. Each gram contains gentamicin sulfate equivalent to 1.0 milligram of gentamicin. Its potency is satisfactory if it is not less than 90 percent and not more than 135 percent of the number of milligrams of gentamicin that it is represented to contain. The gentamicin sulfate used conforms to the standards prescribed therefor by § 444.20(a)(1), except safety.

(2) *Packaging.* In addition to the requirements of § 432.1 of this chapter, it may be dispensed from a pressurized container wherein it is maintained in a compartment separate from the gas used to supply the pressure.

(3) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(4) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The gentamicin sulfate used in making the batch for potency, loss on drying, pH, specific rotation, gentamicins C_1, C_{1a}, and C_2, and identity.

(*b*) The batch for gentamicin potency.

(ii) Samples required:

(*a*) The gentamicin sulfate used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Transfer an accurately weighed representative portion of the cream into a high-speed glass blender. Add 1.0 milliliter of polysorbate 80 and sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration and blend 3 to 5 minutes. Further dilute with solution 3 to the reference concentration of 0.1 microgram of gentamicin per milliliter (estimated).

§ 444.540 Neomycin palmitate dermatologic dosage forms.

§ 444.540a Neomycin palmitate-trypsin-chymotrypsin ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* The drug is an ointment containing in each gram of a suitable and harmless ointment base neomycin palmitate equivalent to 3.5 milligrams of neomycin and 10,000 units of trypsin-chymotrypsin proteolytic activity. The moisture content is not more than 1 percent. The neomycin palmitate used conforms to the requirements of § 444.40(a)(1) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Requests for certification.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin palmitate used in making the batch for potency, moisture pH, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The neomycin palmitate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 444.40(b)(1), except prepare the sample for assay by either of the following methods:

(i) Place approximately 1 gram of the ointment, accurately weighed, in a high-speed glass blender with 1 milliliter of polysorbate 80 and 99 milliliters of absolute methyl alcohol. Blend 3 to 5 minutes. Make proper estimated dilutions in 0.1*M* potassium phosphate buffer, pH 8, to the reference concentration.

(ii) Place an accurately weighed representative portion of the ointment into a separatory funnel containing 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 25 milliliters of 0.1*M* potassium phosphate buffer, pH 8, and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction with new portions of buffer at least three times and any additional times necessary to insure complete extraction of the antibiotic. Combine the extractives and adjust to an appropriate volume to give a stock solution of convenient concentration. Further dilute with 0.1*M* potassium phosphate buffer, pH 8, to the proper, prescribed reference concentration.

The neomycin content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of neomycin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 444.540b Neomycin palmitate-trypsin-chymotrypsin concentrate lotion.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin palmitate-trypsin-chymotrypsin concentrate lotion contains neomycin palmitate and trypsin-chymotrypsin concentrate in a suitable and harmless vehicle. It may contain suitable and harmless preservatives. Each gram of lotion contains neomycin palmitate equivalent to 3.5 milligrams of neomycin and 10,000 units of trypsin-chymotrypsin proteolytic activity. Its moisture content is not more than 1 percent. The neomycin palmitate used conforms to the requirements of § 444.40(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin palmitate used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for neomycin content and moisture.

(ii) Samples required:

(*a*) The neomycin palmitate used in making the batch: 10 packages, each containing 300 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 444.540a(b)(1). The neomycin content is satisfactory if it is not less than 90 percent nor more than 115 percent of the number of milligrams of neomycin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 436.201(b) of this chapter.

§ 444.542 Neomycin sulfate dermatologic dosage forms.

§ 444.542a Neomycin sulfate ointment; neomycin sulfate_____ ointment (the blank being filled in with the established name(s) of the other active ingredient(s) present in accordance with paragraph (a)(1) of this section).

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate ointment contains, in each gram, 3.5 milligrams of neomycin in a suitable and harmless water-soluble or oleaginous ointment base, with or without one or more suitable and harmless dispersants, emollients, and preservatives. The following other drugs may be combined with neomycin sulfate ointment in the indicated amounts, per gram:

(i) If it is for topical use:

(*a*) 0.5 milligram of flurandrenolone; or

(*b*) 0.25 milligram of fluorometholone; or

(*c*) 5.0, 10.0, 15.0, or 25.0 milligrams of hydrocortisone acetate; or

(*d*) 10.0 or 25.0 milligrams of hydrocortisone; or

(*e*) 5.0 milligrams of hydrocortamate hydrochloride; or

(*f*) 1.0, 2.5, or 5.0 milligrams of prednisolone acetate; or

(*g*) 1.0 milligram of triamcinolone acetonide; or

(*h*) 5.0 milligrams of hydrocortisone and 45 milligrams to 55 milligrams of coaltar extract; or

(*i*) 10.0 milligrams of hydrocortisone acetate and 30 milligrams of calcium undecylenate; or

(*j*) 200 milligrams of benzocaine.

(ii) If it is for ophthalmic use:

(a) 5.0 milligrams or 15.0 milligrams of hydrocortisone acetate; or

(b) 2.5 milligrams of sodium prednisolone phosphate; or

(c) 0.5 milligram of sodium dexamethasone phosphate.

(iii) If it is an eye-ear ointment:

(a) 1.0 milligram of methylprednisolone; or

(b) 1.0 milligram of triamcinolone acetonide; or

(c) 5.0 milligrams or 15.0 milligrams of hydrocortisone acetate; or

(d) 2.5 milligrams or 5.0 milligrams of prednisolone acetate.

If it is an oleaginous base, its moisture content is not more than 1.0 percent. If it is intended for ophthalmic use, it is sterile. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1)(i), (vi), and (vii) and, if for ophthalmic use, paragraph (a)(1)(iv) of that section. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* If it contains a steroid or if it is intended for ophthalmic use, it shall be labeled in accordance with the requirements prescribed by § 432.5 of this chapter, and its expiration date is 12 months. If it does not contain a steroid or it is not intended for ophthalmic use each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the label of the immediate container and on the outside wrapper or container, if any:

(a) The batch mark.

(b) The name and quantity of each active ingredient contained in the drug.

(c) An expiration date that is 12 months after the month during which the batch was certified.

(ii) On the label of the immediate container or other labeling attached to or within the package, adequate directions under which the layman can use the drug safely and efficaciously.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The neomycin sulfate used in making the batch for potency, pH, and identity, and, if the ointment is for ophthalmic use, toxicity.

(b) The batch for potency and for moisture if the ointment base is oleaginous and for sterility if the ointment is intended for ophthalmic use.

(ii) Samples required:

(a) The neomycin sulfate used in making the batch: 10 packages each containing approximately 300 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of five immediate containers.

(2) For sterility testing: Twenty immediate containers, collected at regular intervals throughout each filling operation.

(c) In the case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Extraction.* Proceed as directed in § 444.42a(b)(1) of this chapter, except prepare the sample by placing an accurately weighed representative portion of the ointment into a separatory funnel containing 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 25 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0, and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction with new portions of the buffer at least three times and any additional times necessary to insure complete extraction of the antibiotic. Combine the extractives and adjust to an appropriate volume to give a stock solution of convenient concentration. Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0, to the proper prescribed reference concentration.

(ii) *Blending.* Proceed as directed in § 444.42a(b)(1), except prepare the sample for assay as follows: Transfer an accurately weighed sample into a high-speed glass blender, add 1.0 milliliter of polysorbate 80 and sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a stock solution of convenient concentration. Blend 3 to 5 minutes. Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0, to the proper prescribed reference concentration. The content of neomycin is satisfactory if it is not less than 90 percent and not more than 135 percent of the number of milligrams of neomycin that it is represented to contain.

(2) *Sterility.* If the ointment is intended for ophthalmic use, proceed as directed in § 436.20 of this chapter, using the method as described in paragraph (e)(3) of that section.

(3) *Moisture.* If the ointment has an oleaginous base, proceed as directed in § 436.201 of this chapter.

§ 444.542b Neomycin sulfate-_____ cream (the blank being filled in with the established name(s) of the other active ingredient(s) present in accordance with paragraph (a)(1) of this section).

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* The drug is neomycin sulfate and either betamethasone, dexamethasone sodium phosphate, dichlorisone acetate, fluocinolone acetonide, methylprednisolone acetate, or triamcinolone acetonide, with or without one or more suitable and harmless emollients, perfumes, dispersants, and preservatives, in a suitable cream base. It contains, in each gram, 3.5 milligrams of neomycin and one of the following:

(i) 2 milligrams of betamethasone; or

(ii) Dexamethasone sodium phosphate equivalent to 1.0 milligram of dexamethasone phosphate; or

(ii) 1 milligram of sodium dexamethasone phosphate; or

(iii) 2.5 milligrams of dichlorisone acetate; or

(iv) 0.25 milligram of fluocinolone acetonide; or

(v) 2.5 milligrams, 5 milligrams, or 10 milligrams of methylprednisolone acetate; or

(vi) 1 milligram of triamcinolone acetonide.

The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1)(i), (vi), and (vii). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements prescribed by § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The neomycin sulfate used in making the batch for potency, pH, and identity.

(b) The batch for potency.

(ii) Samples required:

(a) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch: A minimum of five immediate containers.

(c) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay; potency.* Proceed as directed in § 444.42a(b)(1), except prepare the sample for assay as follows: Transfer an accurately weighed representative portion into a high-speed glass blender. Add 1.0 milliliter of polysorbate 80 and sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a stock solution of convenient concentration and blend 3 to 5 minutes. Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0, to the proper prescribed reference concentration. Its neomycin content is satisfactory if it is not less than 90 percent nor more than 135 percent of the number of milligrams of neomycin that it is represented to contain.

§ 444.542c Neomycin sulfate-_____ lotion (the blank being filled in with the established name(s) of the other active ingredient(s) present in accordance with paragraph (a)(1) of this section).

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* The drug is a suspension containing, in each milliliter, 3.5 milligrams of neomycin and the following other active ingredients in a suitable and harmless vehicle:

(i) 10 milligrams of diperodon hydrochloride and 7.5 milligrams of aluminum dihydroxy allantoinate; or

(ii) 5 milligrams or 10 milligrams of hydrocortisone acetate; or

(iii) 5 milligrams, 10 milligrams, or 20 milligrams of hydrocortisone; or

(iv) 1 milligram, 2.5 milligrams, or 5 milligrams of prednisolone acetate; or

(v) Prednisolone sodium phosphate equivalent to 5.0 milligrams of prednisolone phosphate; or

(vi) 0.5 milligram of flurandrenolone.

It may also contain one or more suitable and harmless dispersants, emollients,

and preservatives. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1) (i), (vi), and (vii). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* If it contains a corticosteroid, it shall be labeled in accordance with the requirements prescribed by § 432.5 of this chapter and its expiration date is 12 months. If it does not contain a corticosteroid, each package shall bear, on its label or labeling, as hereinafter indicated, the following:

(i) On the label of the immediate container and on the outside wrapper or container, if any:

(*a*) The batch mark.

(*b*) The name and quantity of each active ingredient contained in the drug.

(*c*) An expiration date that is 12 months after the month during which the batch was certified.

(ii) On the label of the immediate container or other labeling attached to or within the package, adequate directions under which the layman can use the drug safely and efficaciously.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, pH, and identity.

(*b*) The batch for potency.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay; potency.* Proceed as directed in § 444.42a(b)(1), except prepare the sample for assay as follows: Place an accurately measured representative portion into a high-speed glass blender with sufficient 0.1*M* potassium phosphate buffer, pH 8, to give a stock solution of convenient concentration. Blend 3 to 5 minutes. Make further dilutions with 0.1*M* potassium phosphate buffer, pH 8, to the proper prescribed reference concentration. Its content of neomycin is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of neomycin that it is represented to contain.

§ 444.542d Neomycin sulfate aerosol.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate aerosol is an aqueous solution of neomycin sulfate equivalent to 397 milligrams of neomycin per 10 ounces of aqueous solution, with or without suitable preservatives, packaged in a pressurized container with inert propellants. It may contain a suitable and harmless perfume. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1) (i), (vi), and (vii). Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such offical compendium.

(2) *Labeling.* In addition to the labeling prescribed by § 432.5 of this chapter each package shall bear on its label or labeling the following as indicated:

(i) On the label of the immediate container and on the outside wrapper or package, if any:

(*a*) The batch mark.

(*b*) The name and quantity of the active ingredient contained in the drug.

(*c*) An expiration date that is 12 months after the month during which the batch was certified.

(ii) On the label of the immediate container: Adequate directions under which the layman can use the drug safely and efficaciously.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, pH, and identity.

(*b*) The batch for potency.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing 300 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay; potency.* Proceed as directed in § 444.42a(b)(1), except prepare the sample for assay as follows: Spray the entire contents of the well-shaken sample into a suitable, tared beaker. Place in a vacuum desiccator, evacuate, and allow to stand at room temperature for 16 to 18 hours. After the gas has volatilized, accurately weigh the beaker and contents and determine the weight of the material in the beaker. Prepare the material for assay by either of the following methods:

(1) Blend an accurately weighed representative portion for 3 to 5 minutes in a high-speed glass blender with 1.0 milliliter of polysorbate 80 and sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a stock solution of convenient concentration. Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0, to the proper prescribed reference concentration.

(2) Quantitatively transfer either an accurately weighed portion of the contents, or the entire contents, from the beaker into a suitable volumetric flask to give a stock solution of convenient concentration. Dilute to volume with 0.1*M* potassium phosphate buffer, pH 8.0. Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0, to the proper prescribed reference concentration.

The content of neomycin sulfate is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of neomycin that it is represented to contain.

§ 444.542e Neomycin sulfate-polymyxin B sulfate ointment.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate-polymyxin B sulfate ointment is an ointment containing, in each gram, 3.5 milligrams of neomycin and 5,000 units of polymyxin B with suitable and harmless emollients, dispersants, and preservatives in a suitable and harmless water-miscible base. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1) (i), (vi), and (vii). The polymyxin B sulfate used conforms to the standards prescribed by § 448.30a(a)(1) (i), (vi), (vii), and (ix) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* Each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the label of the immediate container and on the outside wrapper or container, if any:

(*a*) The batch mark.

(*b*) The name and quantity of each active ingredient contained in the drug.

(*c*) An expiration date that is 12 months after the month during which the batch was certified.

(ii) On the label of the immediate container or other labeling attached to or within the package, adequate directions under which the layman can use the drug safely and efficaciously.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the bach for potency, pH, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, pH, residue on ignition, and identity.

(*c*) The batch for neomycin content and polymyxin content.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The batch: A minimum of 6 immediate containers.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay—*(1) *Potency—*(i) *Neomycin content.* Proceed as directed in § 444.542a(1)(ii). Its content of neomycin is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of neomycin that it is represented to contain.

(ii) *Polymyxin content.* Proceed as directed in § 436.105 of this chapter, except add to each concentration of the polymyxin standard response line a quantity of neomycin to yield the same concentration of neomycin as that present when the sample is diluted to contain 10 units of polymyxin B per milliliter. Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 10 percent potassium phosphate buffer, pH 6.0 (solution 6), and shake well. Remove the buffer layer and repeat the extraction procedure with each of three more 20 to 25 milliliter quantities of solution 6. Combine the extractives in a suitable volumetric flask and fill to volume with solution 6. Further dilute an aliquot with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated). Its content of polymyxin is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of units of polymyxin that it is represented to contain.

§ 444.542f Neomycin sulfate-gramicidin topical ointment; neomycin sulfate-gramicidin-triamcinolone acetonide ointment; neomycin sulfate-gramicidin-fludrocortisone acetate ointment.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Each gram of neomycin sulfate-gramicidin topical ointment contains 2.5 milligrams of neomycin and 0.25 miligram of gramicidin. Neomycin sulfate-gramicidin-triamcinolone acetonide ointment is an ointment containing, in each gram, 2.5 milligrams of neomycin, 0.25 milligram of gramicidin, and 1.0 milligram of triamcinolone acetonide. Neomycin sulfate-gramicidin-fludrocortisone acetate ointment is an ointment containing, in each gram, 2.5 milligrams of neomycin, 0.25 milligram of gramicidin, and 1.0 milligram of fludrocortisone acetate. If it is intended for ophthalmic use, it is sterile. Their moisture content is not more than 1.0 percent. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1) (i), (v), (vi), and (vii), and in addition if it is used in the preparation of an ophthalmic ointment, paragraph (a)(1)(iv) of that section. The gramicidin used conforms to the standards prescribed by § 448.25(a)(1) (i), (iii), (iv), (v), and (vi) of this chapter, and in addition if it is used in the preparation of an ophthalmic ointment, paragraph (a)(1)(ii) of that section. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* If it contains a steroid or it is intended for ophthalmic use, it shall be labeled in accordance with the requirements of § 432.5 of this chapter, and its expiration date is 12 months. If it does not contain a steroid or it is not intended for ophthalmic use, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the label of the immediate container and on the outside wrapper or container, if any:

(*a*) The batch mark.

(*b*) The name and quantity of each active ingredient contained in the drug.

(*c*) An expiration date that is 12 months after the month during which the batch was certified.

(ii) On the label of the immediate container or other labeling attached to or within the package, adequate directions under which the layman can use the drug safely and efficaciously.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity, and in addition, if it is used in the preparation of an ophthalmic ointment, for toxicity.

(*b*) The gramicidin used in making the batch for potency, moisture, residue on ignition, melting point, crystallinity, and identity, and in addition, if it is used in the preparation of an ophthalmic ointment, for toxicity.

(*c*) The batch for neomycin content, gramicidin content, and moisture, and for sterility if it is intended for ophthalmic use.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The gramicidin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*c*) The batch:

(*1*) For all tests except sterility: A minimum of six immediate containers.

(*2*) For sterility testing: Twenty immediate containers, collected at regular intervals throughout each filling operation.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay—*(1) *Potency—*(i) *Neomycin content.* Proceed as directed in § 444.542a(b)(1). Its content of neomycin is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of neomycin that it is represented to contain.

(ii) *Gramicidin content.* Proceed as directed in § 448.25(b)(1) of this chapter, except prepare the sample for assay by the following method: Place an accurately weighed representative portion into a separatory funnel. Dissolve the ointment in 50 milliliters of *n*-hexane. Extract this solution with four 50-milliliter portions of 80 percent alcohol. Collect the extractives in a 250-milliliter volumetric flask, bring to volume with 80 percent alcohol, and mix well. From this stock solution, dilute an aliquot with 95 percent alcohol to reference concentration.

Its content of gramicidin is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of gramicidin that it is represented to contain.

(2) *Sterility.* If the ointment is intended for ophthalmic use, proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(3) of that section. However, if the ointment is not soluble in isopropyl myristate proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except use 100 milligrams in lieu of 300 milligrams of solids.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 444.542g Neomycin sulfate-gramicidin-triamcinolone acetonide cream.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate-gramicidin-triamcinolone acetonide cream is a cream containing 2.5 milligrams of neomycin, 0.25 milligram of gramicidin, and 1.0 milligram of triamcinolone acetonide per gram, with one or more suitable and harmless emollients, dispersants, and preservatives in a suitable and harmless cream base. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1) (i), (vi), and (vii). The gramicidin used conforms to the standards prescribed by § 448.25(a)(1) (i), (iv), (v), and (vi) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., shall conform to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements prescribed by § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, pH, and identity.

(*b*) The gramicidin used in making the batch for potency, residue on ignition, melting point, crystallinity and identity.

(*c*) The batch for neomycin content and gramicidin content.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The gramicidin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*c*) The batch: A minimum of six immediate containers.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay; potency—*(1) *Neomycin content.* Proceed as directed in § 444.542b(b)(1). Its neomycin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of neomycin that it is represented to contain.

(2) *Gramicidin content.* Proceed as directed in § 448.25(b)(1) of this chapter, except to prepare the sample for assay proceed as follows: Place an accurately weighed representative portion into a high-speed glass blender and add sufficient 95 percent alcohol to give a stock solution of convenient concentration. Blend 3 to 5 minutes. Make proper estimated dilutions of an aliquot to the reference concentration with 95 percent alcohol. Its content of gramicidin is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of gramicidin that it is represented to contain.

§ 444.542h Neomycin sulfate-gramicidin-triamcinolone acetonide lotion; neomycin sulfate-gramicidin-fludrocortisone acetate lotion.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality and purity.* The drug is a lotion containing, in each milliliter, 2.5 milligrams of neomycin, 0.25 milligram of gramicidin, and either 1 milligram of triamcinolone acetonide or 1 milligram of fludrocortisone acetate, with one or more suitable and harmless emollients, buffers, dispersants, and preservatives, in a suitable and harmless lotion base. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1)(i), (vi), and (vii). The gramicidin used conforms to the standards prescribed by § 448.25(a)(1)(i), (iv), (v), and (vi) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the requirements prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, pH, and identity.

(*b*) The gramicidin used in making the batch for potency, crystallinity, residue on ignition, melting point, and identity.

(*c*) The batch for neomycin content and gramicidin content.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The gramicidin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*c*) The batch: A minimum of 6 immediate containers.

(*d*) In case of an initial request for certification, each other substance used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay—*(1) *Potency—*(i) *Neomycin content.* Proceed as directed in § 444.542c(b)(1). Its neomycin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of neomycin that it is represented to contain.

(ii) *Gramicidin content.* Proceed as directed in § 448.25(b)(1) of this chapter, except prepare the sample by placing an accurately measured representative portion into a high-speed glass blender with sufficient 95 percent alcohol to give a stock solution of convenient concentration. Blend 3 to 5 minutes. Make proper estimated dilutions in 95 percent alcohol to the reference concentration. Its gramicidin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of gramicidin that it is represented to contain.

§ 444.542i Neomycin sulfate-triamcinolone acetonide topical aerosol; neomycin sulfate-dexamethasone topical aerosol.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* The drug is neomycin sulfate and triamcinolone acetonide or dexamethasone, packaged in a pressurized container with a suitable and harmless vehicle and inert propellants. It contains, in each container, the following:

(i) 26.25 milligrams of neomycin and 7.5 milligrams of triamcinolone acetonide; or

(ii) 52.5 milligrams of neomycin and 15 milligrams of triamcinolone acetonide; or

(iii) 35 milligrams of neomycin and 10 milligrams of dexamethasone.

It may contain one or more suitable and harmless emollients, dispersants, and preservatives. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1)(i), (vi), and (vii). Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assay on:

(*a*) The neomycin sulfate used in making the batch for potency, pH, and identity.

(*b*) The batch for potency.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing 300 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay; potency.* Proceed as directed in § 444.42a(b)(1), except prepare the sample for assay as follows: Spray, according to the directions in the labeling, the entire contents of each container to be tested into a separate 2-liter Erlenmeyer flask held in a horizontal position. Each flask should be fitted with a suitable cover containing an opening large enough to permit entry of the spray into the flask. Add 500 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0, to the flask and shake to dissolve the contents. Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0, to the proper, prescribed reference concentration. The content of neomycin is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of neomycin that it is represented to contain.

§ 444.542j Neomycin sulfate-polymxyin B sulfate-gramicidin-benzocaine ointment.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate-polymyxin B sulfate-gramicidin-benzocaine ointment is neomycin sulfate, polymyxin B sulfate, gramicidin, and benzocaine, with suitable and harmless preservatives, in white petrolatum. Each gram contains 3.5 milligrams of neomycin, 2,000 units of polymyxin B, 0.25 milligram of gramicidin, and 10 milligrams of benzocaine. The moisture content is not more than 1.0 percent. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1)(i), (v), (vi), and (vii). The polymyxin B sulfate used conforms to the standards prescribed by § 448.30a(a)(1)(i), (v), (vi), (vii), and (ix) of this chapter. The gramicidin used conforms to the standards prescribed by § 448.25(a)(1)(i), (iii), (iv), (v), and (vi) of this chapter. Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* Each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the label of the immediate container and on the outside wrapper or container, if any:

(*a*) The batch mark.

(*b*) The name and quantity of each active ingredient contained in the drug.

(*c*) An expiration date that is 12 months after the month during which the batch was certified.

(ii) On the label of the immediate container or other labeling attached to or within the package, adequate directions under which the layman can use the drug safely and efficaciously.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, moisture, pH, residue on ignition, and identity.

(*c*) The gramicidin used in making the batch for potency, moisture, residue on ignition, melting point, crystallinity, and identity.

(d) The batch for neomycin content, polymyxin B content, gramicidin content, and moisture.

(ii) Samples required:

(a) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(c) The gramicidin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(d) The batch: A minimum of seven immediate containers.

(e) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Neomycin content.* Proceed as directed in § 444.542a(b) (1) (i) or (ii). The content of neomycin is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of neomycin that it is represented to contain.

(ii) *Polymyxin B content.* Proceed as directed in § 444.542e(b) (1) (ii) of this chapter. The content of polymyxin B is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of units of polymyxin B that it is represented to contain.

(iii) *Gramicidin content.* Proceed as directed in § 448.25(b) (1) of this chapter, except prepare the sample for assay as follows: Place approximately 1 gram of the ointment, accurately weighed, into a high-speed glass blender. Add that quantity of 95 percent ethyl alcohol which is sufficient to give a stock solution of convenient concentration. Blend 3 to 5 minutes. Make proper estimated dilutions of an aliquot to the reference concentration with 95 percent alcohol. The content of gramicidin is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of gramicidin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

EDITORIAL NOTE: §§ 444.542k, 444.542l, and 444.542m (formerly §§ 1481.30, 1481.30a, and 1481.30b) were revoked at 37 FR 25820, Dec. 5, 1972, to become effective in 40 days. Objections and a request for hearing were received by the Commissioner of Food and Drugs.

Pending a review of the objections, the Commissioner has determined that the effective date of the revocations be postponed. See 38 FR 7007, Mar. 15, 1973. For convenience of the user the revoked sections are set forth below in their entirety.

§ 444.542k Neomycin sulfate (commercial grade)-aluminum chlorohydroxide cream deodorant.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate (commercial grade)-aluminum chlorohydroxide cream deodorant is neomycin sulfate (commercial grade) and aluminum chlorohydroxide in a suitable cream base. It contains, in each gram, the following:

(i) 1 milligram of neomycin and 150 milligrams of aluminum chlorohydroxide; or

(ii) 1.75 milligrams of neomycin and either 121 milligrams or 213 milligrams of aluminum chlorohydroxide.

It may also contain one or more suitable emollients, perfumes, colorings, dispersants, solubilizers, buffers, and preservatives. The neomycin sulfate (commercial grade) used conforms to the standards prescribed by § 444.42b(a) (1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* Each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the label of the immediate container and on the outside wrapper or container, if any:

(a) The batch mark.

(b) The name and quantity of each active ingredient contained in the drug.

(c) An expiration date that is 12 months after the month during which the batch was certified, unless the use of a longer expiration period has been approved in accordance with the provisions of § 432.5(a) (3) of this chapter.

(ii) On the label of the immediate container or other labeling attached to or within the package, adequate directions for lay use.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The neomycin sulfate (commercial grade) used in making the batch for potency, toxicity, moisture, pH, and identity.

(b) The batch for potency.

(ii) Samples required:

(a) The neomycin sulfate (commercial grade) used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch: A minimum of five immediate containers.

(c) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay; potency.* Proceed as directed in § 444.42a(b) (1), except prepare the sample for assay as follows: Weigh accurately about 3 grams into a 15-milliliter centrifuge tube, cap the tube with aluminum foil, and heat over a steam bath until the cream is liquefied. Add an accurately measured volume of from 7 to 9.5 milliliters of a 10 percent solution of ammonia. Stir thoroughly to allow the heavy precipitate of aluminum hydroxide to form. Blend the contents for at least 3 minutes in a suitable homogenizer. Centrifuge for 20 minutes at 3000 revolutions per minute. Remove 0.5 to 1.0 milliliter of the clear supernatant and evaporate to dryness under a stream of air. Dissolve and dilute the residue with 0.1*M* potassium phosphate buffer, pH 8.0, to the proper prescribed reference concentration of neomycin. The potency is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of neomycin that the product is represented to contain.

§ 444.542l Neomycin sulfate (commercial grade)-aluminum chlorohydroxide deodorant lotion; neomycin sulfate (commercial grade)-aluminum chlorohydroxide-aluminum chloride deodorant lotion.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate (commercial grade)-aluminum chlorohydroxide deodorant lotion contains neomycin sulfate (commercial grade) and aluminum chlorohydroxide in a suitable lotion vehicle. It contains, in each milliliter, the following:

(i) 1.96 milligrams of neomycin and 135 milligrams of aluminum chlorohydroxide; or

(ii) 3.98 milligrams of neomycin and either 199 milligrams or 217 milligrams of aluminum chlorohydroxide; or

(iii) 1.08 milligrams of neomycin and 217 milligrams of aluminum chlorohydroxide.

Neomycin sulfate (commercial grade)-aluminum chlorohydroxide-aluminum chloride deodorant lotion contains, in each milliliter, 1.96 milligrams of aluminum chlorohydroxide, and 11.2 milligrams of aluminum chloride. The preparations may also contain one or more suitable emollients, perfumes, colorings, dispursants, solubulizers, buffers, and preservatives in an aqueous vehicle. Their pH is not less than 3.5 and not more than 6.5. The neomycin sulfate (commercial grade) used conforms to the standards of § 444.42b(a) (1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* Each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the label of the immediate container and on the outside wrapper or container, if any:

(a) The batch mark.

(b) The name and quantity of each active ingredient contained in the drug.

(c) An expiration date that is 12 months after the month during which the batch was certified, unless the use of a longer expiration period has been approved in accordance with the provisions of § 432.5(a) (3) of this chapter.

(ii) On the label of the immediate container or other labeling attached on or within the package, adequate directions for lay use.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The neomycin sulfate (commercial grade) used in making the batch for potency, toxicity, moisture, pH, and identity.

(b) The batch for potency and pH.

(ii) Samples required:

(a) The neomycin sulfate (commercial grade) used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch: A minimum of five immediate containers.

(c) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 444.42a(b) (1), except prepare the sample for assay as follows: Remove a 3.0-milliliter portion with a suitable syringe and transfer to a 15-milliliter centrifuge tube. Add an accurately measured volume of from 7 to 9.5 milliliters of a 10 percent solution of ammonia. Stir thoroughly to allow the heavy precipitate of aluminum hydroxide to form. Blend the contents for at least 3 minutes in a suitable homogenizer. Centrifuge for 20 minutes at 3000 revolutions per minute. Remove 0.5 to 1.0 milliliter of the clear supernatant and evaporate to dryness under a stream of air. Dissolve and dilute the residue with 0.1*M* potassium phosphate buffer, pH 8.0, to the proper prescribed reference concentration of neomycin. The potency is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of neomycin that the product is represented to contain.

(2) *pH.* Proceed as directed in § 440.80a(b) (5) (ii) of this chapter, using the undiluted sample.

§ 444.542m Neomycin sulfate-aluminum chlorohydroxide deodorant lotion.

Neomycin sulfate-aluminum chlorohydroxide deodorant lotion conforms to all require-

ments and is subject to all procedures prescribed by § 444.542l for neomycin sulfate (commercial grade)-aluminum chlorohydroxide deodorant lotion except that:

(a) Each milliliter contains 3.5 milligrams of neomycin and 200 milligrams of aluminum chlorohydroxide.

(b) The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a) (1) (i), (v), (vi), and (vii).

§ 444.570 Streptomycin dermatologic dosage forms.

§ 444.570a Streptomycin ointment; dihydrostreptomycin ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Streptomycin ointment and dihydrostreptomycin ointment are streptomycin or dihydrostreptomycin in a suitable and harmless ointment base, with or without suitable and harmless dispersing and suspending agents and preservatives. Their potency is not less than 5,000 μg. per gram of ointment. The streptomycin used conforms to the requirements of § 444.70a(a) (1), except § 444.70a(a) (1) (ii), (iii), (iv), (v), and (vi). The dihydrostreptomycin used conforms to the requirements of § 444.10a (a), except the standards for sterility, toxicity, pyrogens, histamine, and moisture. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* Streptomycin ointment and dihydrostreptomycin ointment shall be packaged in collapsible tubes which shall be well-closed containers as defined by the U. S. P., and each such tube shall not be larger than the 2-ounce size, except if it is labeled solely for hospital use it may be packaged in immediate containers of glass which meet the test for tight containers as defined by the U. S. P. The composition of the immediate container and closure shall be such as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106 (b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its outside wrapper or container and the immediate container the statement "Expiration date ----------", the blank being filled in with the date that is 24 months after the month during which the batch was certified.

(4) *Requests for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch of streptomycin ointment or dihydrostreptomycin ointment shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless it was previously submitted) the date on which the latest assay of the streptomycin used in making such batch was completed, the quantity of each ingredient used in making the batch, the date on which the latest assay of the drug comprising such batch was completed, and that each component of the ointment base used conforms to the requirements prescribed therefor, if any, by this section.

(ii) Except as otherwise provided by paragraph (a) (4) (iv) of this section such person shall submit in connection with his request results of the tests and assays listed after each of the following, made by him on an accurately representative sample of:

(*a*) The batch; potency.

(*b*) The streptomycin or dihydrostreptomycin used in making the batch; potency, pH, streptomycin content if it is dihydrostreptomycin and crystallinity if it is crystalline dihydrostreptomycin sulfate.

(iii) Except as otherwise provided by paragraph (a) (4) (iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch; one immediate container for each 5,000 immediate containers in the batch, but in no case less than 5 immediate containers unless each such container is packaged for hospital use and contains more than 2 ounces, in which case the sample shall consist of approximately 1 ounce of ointment for each 5,000 immediate containers in the batch, but in no case less than five 1-ounce portions. Such sample shall be collected by taking single immediate containers or 1-ounce portions at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(*b*) The streptomycin or dihydrostreptomycin used in making the batch; 5 packages containing approximately equal portions of not less than 0.5 gram each, packaged in accordance with the requirements of § 444.70a(a) (2).

(*c*) In case of an initial request for certification, the ingredients used in making the ointment base of the batch; one package of each containing approximately 200 grams, except for the suspending and dispersing agents used, in which case the sample shall consist of approximately 5 grams.

(iv) No result referred to in paragraph (a) (4) (ii) (*b*) of this section, and no sample referred to in paragraph (a) (4) (iii) (*b*) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay*—(1) *Streptomycin content.* Proceed as directed in § 444.70a(b) (1) except paragraphs (b) (1) (x) and (xi) thereof, and in lieu of the directions in § 444.70a(b) (1) (v), prepare the sample as follows: Accurately weigh the tube and contents and squeeze approximately 1.0 gram into a blending jar containing 50 milliliters of 0.10*M* potassium phosphate buffer (pH 7.8 to 8.0). Reweigh the tube to obtain weight of ointment used in the test. Using a high-speed blender, blend the mixture for 3 minutes. Dilute an aliquot of the mixture to contain 100 micrograms of streptomycin base (estimated) per milliliter. Transfer 1.0 milliliter of this solution to a 100-milliliter flask and make up to volume with 0.10*M* potassium phosphate buffer (pH 7.8 to 8.0). Use this last dilution in the assay for potency. The potency of streptomycin ointment is satisfactory if it contains not less than 85 percent of the number of micrograms of streptomycin base per gram it is represented to contain.

(2) *Dihydrostreptomycin content.* Proceed as directed in paragraph (b) (1) of this section, using the dihydrostreptomycin working standard as a standard of comparison. Its content of dihydrostreptomycin is satisfactory if it contains not less than 85 percent of the number of micrograms of dihydrostreptomycin base per gram it is represented to contain.

§ 444.570b Streptomycin for topical use; streptomycin with ------ (the blank being filled in with the name of the vehicle if a package combination) for topical use.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Streptomycin for topical use conforms to all the requirements prescribed by § 444.70a(a) (1) for streptomycin, and may be packaged in combination with a container of a suitable and harmless vehicle.

(2) *Packaging.* The immediate container of streptomycin for topical use shall be of colorless transparent glass so closed as to be a tight container as defined by the U.S.P., shall be sterile at the time of filling and closing, shall be so sealed that its contents cannot be used without destroying such seal, and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded. Each such container shall contain not less than 20 milligrams.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106 (b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its outside label or labeling, as hereinafter indicated, the following:

(i) On the outside wrapper or container and the immediate container, the statement, "Expiration date ----------", the blank being filled in with the date that is 48 months after the month during which the batch was certified.

(ii) If it is a packaged combination, on the immediate container of the vehicle in the combination, a statement giving the method of dissolving the streptomycin in the vehicle and the statement "The solution may be stored at room temperature for 1 week without significant loss of potency".

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certifica-

tion of a batch of streptomycin for topical use shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the number of milligrams in each package, and (unless it was previously submitted) the date on which the latest assay of the drug comprising the batch was completed. Such request shall be accompanied or followed by the results of tests and assays made by him on the batch for potency, sterility, toxicity, pyrogens, histamine content, moisture, and pH. If such batch, or any part thereof, is to be packaged with a vehicle, such request shall be accompanied by a statement that such vehicle conforms to the requirements prescribed therefor by this section.

(ii) Such person shall submit in connection with his request an accurately representative sample of the batch, consisting of the following:

(*a*) For all tests except sterility: One immediate container for each 5,000 immediate containers in such batch; but in no case less than 50 immediate containers.

Such samples shall be collected by taking single immediate containers, before or after labeling, at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(*b*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(iii) In case of an initial request for the certification of a batch of streptomycin for topical use which is to be packaged in combination with a container of a vehicle, or when any change is made in the composition of such vehicle, such person shall submit in connection with his request five packages of the vehicle included in the combination.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 444.70a (b) (1), except paragraph (b) (1) (xi) thereof. The potency of streptomycin for topical use is satisfactory if the immediate containers are represented to contain:

(i) Less than 500 milligrams and contain 85% or more of the number of milligrams so represented;

(ii) More than 500 milligrams and contain 90% or more of the number of milligrams so represented.

(2) *Sterility, toxicity, pyrogens, histamine, moisture, pH.* Proceed as directed in § 444.70a(b) (2) through (6).

§ 444.570c Streptomycin-bacitracin-polymyxin gauze pads.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Streptomycin-bacitracin-polymyxin gauze pads are absorbent gauze that is impregnated with streptomycin, bacitracin, and polymyxin B. Each square inch of such pad shall contain not less than 1 milligram of streptomycin, 50 units of bacitracin, and 1,000 units of polymyxin B. It is sterile. Its moisture content is not more than 5 percent. The streptomycin used conforms to the standards prescribed therefor by § 444.70a(a)(1), except paragraph (a)(1) (ii), (iv), and (v) of that section. The bacitracin used conforms to the standards prescribed therefor by § 448.10a(a)(1), except § 448.10a(a)(1) (ii), (iv), and (viii). The polymyxin used conforms to the standards prescribed therefor by § 444.170a(a)(1). The absorbent gauze used conforms to the standards prescribed therefor by the U.S.P.

(2) *Packaging.* Unless it is intended solely for hospital use, and is conspicuously so labeled, each streptomycin-bacitracin-polymyxin gauze pad shall be packaged individually. In all cases the immediate container shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* Each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the outside wrapper or container and the immediate container:

(*a*) The batch mark;

(*b*) The number of milligrams of streptomycin in each pad of the batch;

(*c*) The number of units of bacitracin in each pad of the batch;

(*d*) The number of units of polymyxin B in each pad of the batch;

(*e*) The length, width, and type of gauze contained in the pad; and

(*f*) The statement "Expiration date ----------", the blank being filled in with the date which is 12 months after the month during which the batch was certified, except that the blank may be filled in with the date which is 24 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time such drug as prepared by him complies with the standards prescribed by paragraph (a) (1) of this section: *Provided, however,* That such expiration date may be omitted from the immediate container if such immediate container is packaged in an individual wrapper or container.

(ii) On the immediate container, the statement "Sterility of the pad cannot be guaranteed if the package bears evidence of damage or has been previously opened".

(iii) On the circular or other labeling within or attached to the package, directions and precautions adequate for the use of such pads.

(4) *Request for certification; samples.* (i) In addition to complying with requirements of § 431.1 of this chapter, a person who requests certification of a batch of streptomycin-bacitracin-polymyxin gauze pads shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch marks and (unless they were previously submitted) the dates on which the latest assays of the streptomycin bacitracin, and polymyxin used in making such batch were completed, the potency of each pad in the batch, the quantity of each ingredient used in making the batch, the date on which the latest assay of the drug comprising such batch was completed, and a statement that the absorbent gauze used in making the batch conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided in paragraph (a) (4) (iv) of this section, such person shall submit in connection with his request results of the tests and assays listed after each of the following, made by him on an accurately representative sample of:

(*a*) The batch; average potency of streptomycin, bacitracin, and polymyxin per pad, sterility, and average moisture.

(*b*) The streptomycin used in making the batch; potency, toxicity, moisture, pH, and ash content.

(*c*) The bacitracin used in making the batch; potency, toxicity, moisture, and pH.

(*d*) The polymyxin used in making the batch; potency and toxicity.

(iii) Except as otherwise provided by paragraph (a) (4) (iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch:

(*1*) For all tests except sterility: one pad for each 5,000 pads in the batch, but in no case less than 30 pads.

(2) For sterility testing: 20 pads.

Such samples shall be collected by taking single pads at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(*b*) The streptomycin used in making the batch; six packages, each containing approximately equal portions of not less than 0.5 gram, packaged in accordance with the requirements of § 444.70a(a)(2).

(*c*) The bacitracin used in making the batch; six packages, each containing approximately equal portions of not less than 0.5 gram, packaged in accordance with the requirement of § 448.10a(a)(2) of this chapter.

(*d*) The polymyxin used in making the batch; five packages, each containing approximately equal portions of not less than 0.5 gram.

(*e*) In case of an initial request for certification, one package consisting of approximately 5 linear yards, or the equivalent, of the absorbent gauze used.

(iv) No result referred to in paragraph (a) (4) (ii) (*b*), (*c*), and (*d*) of this section, and no sample referred to in paragraph (a) (4) (iii) (*b*), (*c*), and (*d*) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 444.170a (b) (1) (i), except soak the sample for not less than 1 hour with frequent agitation.

(2) *Sterility.* Using the entire gauze pad when possible or the largest portion that can be inserted into the culture tube, proceed as directed in § 436.20 of

this chapter, using the method described in paragraph (e)(2) of that section.

(3) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) of this chapter.

Subpart G—Vaginal Dosage Forms

§ 444.642a Neomycin sulfate-polymyxin B sulfate-acetarsone vaginal suppositories.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate-polymyxin B sulfate-acetarsone vaginal suppositories are suppositories composed of neomycin sulfate, polymyxin B sulfate, and acetarsone, with one or more suitable and harmless binders, lubricants, and dispersants in a suitable and harmless base. Each suppository contains 3.5 milligrams of neomycin, 20,000 units of polymyxin B, and 250 milligrams of acetarsone. Its moisture content is not more than 5.0 percent. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1)(i), (v), (vi), and (vii). The polymyxin B sulfate used conforms to the standards prescribed by § 448.30a(a)(1)(i), (v), (vi), (vii), and (ix) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, moisture, pH, residue on ignition, and identity.

(*c*) The batch for neomycin content, polymyxin content, and moisture.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The batch: A minimum of 30 suppositories.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay—*(1) *Potency—*(i) *Neomycin content.* Proceed as directed in § 444.42a(b)(1), except prepare the sample for assay as follows: Place a representative number of suppositories into a high-speed glass blender with 1.0 milliliter of polysorbate 80 and sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a stock solution of convenient concentration. Blend 3 to 5 minutes. Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0, to the proper prescribed reference concentration. The content of neomycin is satisfactory if it contains not less than 90 percent and not more than 130 percent of the number of milligrams of neomycin that it is represented to contain.

(ii) *Polymyxin content.* Proceed as directed in § 448.30a(b)(1) of this chapter, except add to each concentration of the polymyxin standard curve a quantity of neomycin to yield the same concentration of neomycin as that present when the sample is diluted to contain 10 units of polymyxin per milliliter, and prepare the sample for assay as follows: Blend a representative number of suppositories in a high-speed glass blender, with 1.0 milliliter of polysorbate 80 and sufficient 10 percent potassium phosphate buffer, pH 6.0, to give a stock solution of convenient concentration. Further dilute with 10 percent potassium phosphate buffer, pH 6.0, to the reference concentration of 10 units of polymyxin per milliliter. Its content of polymyxin is satisfactory if it contains not less than 90 percent and not more than 130 percent of the number of units of polymyxin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

Subparts H and I—[Reserved]

Subpart J—Certain Other Dosage Forms

§ 444.942 Neomycin sulfate in certain other dosage forms.

§ 444.942a Neomycin sulfate for prescription compounding.

(a) *Requirements for certification.* (1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate for prescription compounding is the sulfate salt of a kind of neomycin or a mixture of two or more such salts. It is so purified and dried that:

(i) It has a potency of not less than 600 micrograms of neomycin per milligram.

(ii) It is nontoxic.

(iii) Its moisture content is not more than 8 percent.

(iv) Its pH is an aqueous solution containing 33 milligrams per milliliter is not less than 5.0 nor more than 7.5.

(v) It gives a positive identity test for neomycin.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. Each such container shall contain not less than 10 grams and not more than 100 grams of neomycin sulfate.

(3) *Labeling.* Each package shall bear on its outside wrapper or container and on the immediate container the following:

(i) The statements "For prescription compounding" and "Caution: Federal law prohibits dispensing without prescription".

(ii) The statement "Not sterile".

(iii) The batch mark.

(iv) The number of milligrams of neomycin activity in each gram of the neomycin sulfate.

(v) The statement "Expiration date _____", the blank being filled in with the date that is 12 months after the month during which the batch was certified.

(vi) The number of grams of neomycin sulfate in each immediate container of the batch.

(4) *Requests for certification; samples.* (i) In addition to complying with the conditions of § 431.1 of this chapter, a person who requests certification of a batch of neomycin sulfate for prescription compounding shall submit with his request a statement showing the batch mark, the number of packages of each size in the batch, and the date on which the latest assay of the drug comprising such batch was completed. Such request shall be accompanied or followed by results of tests and assays made by him on the batch for potency, toxicity, moisture, pH, and identity.

(ii) Such person shall submit with his request a sample consisting of a 0.5 gram portion for each 5,000 packages in the batch, but in no case less than 10 such portions. Each such portion shall be collected at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(b) *Tests and methods of assay; potency, toxicity, moisture, pH, and identity.* Proceed as directed in § 444.42a(b)(1), (4), (5), (6), and (7).

§ 444.942b Sterile neomycin sulfate and polymyxin B sulfate solution.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Sterile neomycin sulfate and polymyxin B sulfate solution is an aqueous solution containing in each milliliter 40 milligrams of neomycin and 200,000 units of polymyxin B. It is sterile. Its pH is not less than 5.0 and not more than 6.0. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1)(i), (iv), (vi), and (vii). The polymyxin B sulfate used conforms to the standards prescribed by § 448.30a(a)(1)(i), (iv), (vi), (vii), and (ix) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or the N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* In addition to being labeled in accordance with the requirements of § 432.5 of this chapter, the labeling shall include a statement to the effect that the drug is to be diluted for use as a urinary bladder irrigant and is not for injection. Its expiration date is 12 months.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, toxicity, pH, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, toxicity, pH, residue on ignition, and identity.

(*c*) The batch for neomycin content, polymyxin B content, pH, and sterility.

(ii) Samples required:

(a) The neomycin sulfate used in making the batch: Ten packages, each containing approximately 300 milligrams.

(b) The polymyxin B sulfate used in making the batch: Ten packages, each containing approximately 300 milligrams.

(c) The batch:

(1) For all tests except sterility: A minimum of six immediate containers.

(2) For sterility testing: Twenty immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Neomycin content.* Proceed as directed in § 444.42a(b)(1), except prepare the sample as follows: Remove an accurately measured portion and dilute with 0.1*M* potassium phosphate buffer, pH 8.0, to the proper prescribed reference concentration. The neomycin content is satisfactory if it is not less than 90 percent nor more than 130 percent of the number of milligrams of neomycin that it is represented to contain.

(ii) *Polymyxin B content.* Remove an accurately measured portion and dilute with 10-percent potassium phosphate buffer, pH 6.0, to a reference concentration of 10 units of polymyxin B per milliliter. Proceed as directed in § 448.30a(b)(1) of this chapter, except add to each concentration of the polymyxin B standard curve a quantity of neomycin to yield the same concentration of neomycin as that present when the sample is diluted to contain 10 units of polymyxin B per milliliter. The polymyxin B content is satisfactory if it is not less than 90 percent nor more than 130 percent of the number of units of polymyxin B that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using the undiluted sample.

PART 446—TETRACYCLINE ANTIBIOTIC DRUGS

Subpart A—Bulk Drugs

Sec.
446.10a Sterile chlortetracycline hydrochloride.
446.15 Demeclocycline.
446.16 Demeclocycline hydrochloride.
446.20 Doxycycline hyclate.
446.20a Sterile doxycycline hyclate.
446.21 Doxycycline monohydrate.
446.50 Methacycline hydrochloride.
446.60 Minocycline hydrochloride.
446.65a Sterile oxytetracycline.
446.66 Oxytetracycline calcium.
446.67a Sterile oxytetracycline hydrochloride.
446.75a Sterile rolitetracycline.
446.76a Sterile rolitetracycline nitrate.
446.80 Tetracycline.
446.81a Sterile tetracycline hydrochloride.
446.82 Tetracycline phosphate complex.

Subpart B—Oral Dosage Forms

446.110 Chlortetracycline hydrochloride oral dosage forms.
446.110a Chlortetracycline hydrochloride tablets; tetracycline hydrochloride tablets; tetracycline tablets.
446.110b Chlortetracycline hydrochloride capsules; tetracycline hydrochloride capsules; tetracycline capsules; tetracycline phosphate complex capsules.
446.111 Chlortetracycline calcium sirup (chlortetracycline calcium oral drops); tetracycline sirup (tetracycline oral drops); tetracycline magnesium sirup (tetracycline magnesium oral drops).
446.115 Demeclocycline oral dosage forms.
446.115a Demeclocycline oral suspension.
446.115b Demeclocycline for oral suspension.
446.115c Demeclocycline-nystatin for oral suspension.
446.116 Demeclocycline hydrochloride oral dosage forms.
446.116a Demeclocycline hydrochloride tablets.
446.116b Demeclocycline hydrochloride-nystatin tablets.
446.116c Demeclocycline hydrochloride capsules.
446.116d Demeclocycline hydrochloride-nystatin capsules.
446.120 Doxycycline hyclate capsules.
446.121 Doxycycline monohydrate for oral suspension.
446.150 Methacycline hydrochloride oral dosage forms.
446.150a Methacycline hydrochloride capsules.
446.150b Methacycline hydrochloride sirup.
446.160 Minocycline hydrochloride oral dosage forms.
446.160a Minocycline hydrochloride tablets.
446.160b Minocycline hydrochloride capsules.
446.160c Minocycline hydrochloride oral suspension.
446.165 Oxytetracycline oral dosage forms.
446.165a Oxtetracycline tablets.
446.165b Oxytetracycline capsules; oxytetracycline hydrochloride capsules.
446.165c Oxytetracycline-nystatin capsules.
446.165d Oxytetracycline for oral suspension.
446.165e Oxytetracycline-nystatin for oral suspension.
446.166 Calcium oxytetracycline sirup.
446.167 Oxytetracycline hydrochloride soluble tablets.
446.180 Tetracycline oral dosage forms.
446.180a Tetracycline-nystatin oral suspension; tetracycline phosphate complex-nystatin oral suspension (tetracycline phosphate complex-nystatin oral drops).
446.180b Tetracycline-nystatin for oral suspension.
446.181 Tetracycline hydrochloride oral dosage forms.
446.181a Tetracycline hydrochloride-nystatin tablets.
446.181b Tetracycline hydrochloride-nystatin capsules; tetracycline phosphate complex-nystatin capsules.
446.181c Tetracycline hydrochloride oral suspension (tetracycline hydrochloride homogenized mixture); tetracycline phosphate complex oral suspension (tetracycline phosphate complex oral drops); tetracycline hydrochloride oral solution; tetracycline calcium oral suspension; tetracycline oral suspension.

Subpart C—Injectable Dosage Forms

446.210 Sterile chlortetracycline hydrochloride.
446.220 Doxycycline hyclate for injection.
446.260 Sterile minocycline hydrochloride.
446.265 Oxytetracycline intramuscular solution.
446.267 Oxytetracycline hydrochloride for injection.
446.275 Rolitetracycline injectable dosage forms.
446.275a Rolitetracycline for intravenous use.
446.275b Rolitetracycline for intramuscular use.
446.276 Rolitetracycline nitrate injectable dosage forms.
446.276a Rolitetracycline nitrate for intravenous use.
446.276b Rolitetracycline nitrate for intramuscular use.
446.281 Tetracycline hydrochloride injectable dosage forms.
446.281a Sterile tetracycline hydrochloride.
446.281b Tetracycline hydrochloride for intramuscular use; tetracycline phosphate complex for intramuscular use.

Subpart D—Ophthalmic Dosage Forms

446.310 Chlortetracycline hydrochloride ophthalmic dosage forms.
446.310a Chlortetracycline ophthalmic (chlortetracycline hydrochloride ophthalmic); tetracycline hydrochloride ophthalmic.
446.310b Chlortetracycline hydrochloride ointment; chlortetracycline calcium ointment; chlortetracycline calcium cream; tetracycline hydrochloride ointment (tetracycline hydrochloride in oil suspension); tetracycline ointment (tetracycline cream).
446.367 Oxytetracycline hydrochloride ophthalmic dosage forms.
446.367a Ophthalmic oxytetracycline hydrochloride.
446.367b Oxytetracycline hydrochloride ophthalmic oil suspension.
446.367c Oxytetracycline hydrochloride-hydrocortisone acetate ophthalmic and otic suspension.
446.367d Oxytetracycline hydrochloride ophthalmic ointment.
446.367e Oxytetracycline hydrochloride-polymyxin B sulfate eye and ear ointment.

Subpart E—Otic Dosage Forms

446.467 Oxytetracycline hydrochloride otic dosage forms.
446.467a Oxytetracycline hydrochloride-polymyxin B sulfate-benzocaine for otic solution.
446.467b Oxytetracycline hydrochloride-polymyxin B sulfate eye and ear ointment.
446.467c Oxytetracycline hydrochloride-hydrocortisone acetate ophthalmic and otic suspension.
446.481 Tetracycline hydrochloride otic (tetracycline hydrochloride for ear solution).

Subpart F—Dermatologic Dosage Forms

446.510 Chlortetracycline dermatologic dosage forms.
446.510a Chlortetracycline hydrochloride ointment; chlortetracycline calcium ointment; chlortetracycline calcium cream; tetracycline hydrochloride ointment (tetracycline hydrochloride in oil suspension); tetracycline ointment (tetracycline cream).
446.510b Chlortetracycline powder (chlortetracycline hydrochloride powder); tetracycline hydrochloride powder; tetracycline powder.
446.567 Oxytetracycline hydrochloride dermatologic dosage forms.

Sec.
446.567a Oxytetracycline hydrochloride-hydrocortisone topical ointment.
446.567b Oxytetracycline hydrochloride-polymyxin B sulfate topical ointment.
446.567c Oxytetracycline hydrochloride-polymyxin B sulfate topical powder.
446.567d Oxytetracycline hydrochloride-hydrocortisone aerosol topical.
446.567e Oxytetracycline hydrochloride-polymyxin B sulfate-hydrocortisone aerosol topical.
446.581 Tetracycline hydrochloride dermatologic dosage forms.
446.581a Tetracycline hydrochloride-neomycin spray ointment topical.
446.581b Tetracycline hydrochloride-neomycin in oil suspension.

Subpart G—Vaginal Dosage Forms

446.610 Chlortetracycline suppositories (chlortetracycline hydrochloride suppositories); tetracycline hydrochloride suppositories.
446.667 Oxytetracycline hydrochloride-polymyxin B sulfate vaginal tablets.

Subpart H—Rectal Dosage Forms

446.710 Chlortetracycline suppositories (chlortetracycline hydrochloride suppositories); tetracycline hydrochloride suppositories.

Subpart I—[Reserved]

Subpart J—Certain Other Dosage Forms

446.910 Chlortetracycline hydrochloride impregnated surgical silk.

AUTHORITY: Sec. 507, 59 Stat. 463, as amended (21 U.S.C. 357).

Subpart A—Bulk Drugs

§ 446.10a Sterile chlortetracycline hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chlortetracycline hydrochloride is the yellow hydrochloride salt or crystalline hydrochloride salt of a kind of chlortetracycline or a mixture of two or more such salts with or without one or more suitable and harmless buffer substances if it is intended for intravenous use. It is so purified and dried that:

(i) Its potency, and the potency of the chlortetracycline used in the manufacture of chlortetracycline for intravenous use, is not less than 900 micrograms per milligram;

(ii) It is sterile;

(iii) It is nontoxic;

(iv) It is nonpyrogenic;

(v) It contains no histamine nor histamine-like substances;

(vi) Its moisture content is not more than 2.0 percent;

(vii) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.3 and not more than 3.3, except if it is intended for intravenous use and it contains buffer substances its pH is not less than 8 and not more than 9.5.

(2) *Packaging.* In all cases the immediate containers shall be tight containers as defined by the U. S. P., shall be sterile at the time of filling and closing, shall be so sealed that the contents cannot be used without destroying the seal, and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded. In case it is intended for intravenous use, it shall be packaged in immediate containers of colorless, transparent glass, closed by a substance through which a hypodermic needle may be introduced and withdrawn without removing the closure or destroying its effectiveness; each such container shall contain not more than 1.0 gram and each shall contain one or more suitable and harmless buffer substances, or each shall be packaged in combination with a container of a suitable and harmless diluent.

(3) *Labeling.* (i) *It is packaged for dispensing.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its label or labeling, as hereinafter indicated, the following:

(*a*) On the outside wrapper or container and the immediate container, the statement "Expiration date _____", the blank being filled in with the date that is 12 months, or if it is crystalline chlortetracycline 60 months, after the month during which the batch was certified, except that if it is crystalline chlortetracycline and it contains buffer substances the blank shall be filled in with the date that is 36 months after the month during which the batch was certified.

(*b*) On the circular or other labeling within or attached to the package, a statement of the conditions under which such solutions should be stored, including a reference to their instability when stored under other conditions, and a statement, "Sterile solutions must be injected immediately after preparation".

(ii) *It is packaged solely for manufacturing use and/or repacking.* Each package shall bear on its outside wrapper or container and the immediate container the following:

(*a*) The potency per milligram and the number of grams in the immediate container.

(*b*) The statement "Caution: Federal law prohibits dispensing without prescription".

(*c*) The statement "For manufacturing use", "For repacking", or "For manufacturing use or repacking".

(*d*) The information required by paragraph (a)(3)(i)(*a*) of this section.

(4) *Request for certification, check tests and assays; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in the batch, the number of milligrams in each package, and (unless it was previously submitted) the date on which the latest assay of the drug comprising the batch was completed; and if it is intended for intravenous use and contains buffer substances, the date and the results of the latest test for potency of the chlortetracycline used in making such batch. Such request shall be accompanied or followed by the results of tests and assays made by him on the batch for potency, sterility, toxicity, pyrogens, histamine content, crystallinity (if it is the crystalline salt), moisture, and pH. If such batch or any part thereof is to be packaged with a solvent such request shall also be accompanied by a statement that such solvent conforms to the requirements prescribed therefor by this section.

(ii) If such batch is packaged for dispensing, such person shall submit with his request an accurately representative sample of the batch, consisting of the following:

(*a*) For all tests except sterility: One immediate container for each 5,000 immediate containers in such batch, but in no case less than eight immediate containers, collected by taking single immediate containers at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(*b*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(iii) If such batch is packaged for repacking or for use as an ingredient in the manufacture of another drug, such person shall submit with his request an accurately representative sample of the batch, consisting of the following:

(*a*) For all tests except sterility; 5 packages, each containing approximately 0.5 gram.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

Each such package shall be packaged in accordance with the requirements of paragraph (a)(2) of this section.

(iv) In connection with contemplated requests for certification of repacked batches or batches of another drug in the manufacture of which it is to be used, the manufacturer of a batch which is to be so repacked or used may request the Commissioner to make check tests and assays on a sample of such batch, taken as prescribed by paragraph (a)(4)(iii) of this section. From the information required by paragraph (a)(4)(i) of this section may be omitted results of tests and assays not required for the batch when used in such other drug. The Commissioner shall report to such manufacturer results of such check tests and assays as are so requested.

(v) If such batch is intended for intravenous use and it contains buffer substances, such person shall submit in connection with his request (unless it has been previously submitted) one immediate container containing approximately 0.5 gram of the chlortetracycline used in making such batch.

(b) *Tests and methods of assay*—(1) *Potency.* (i) Cylinders (cups). Use cylinders described in § 440.80a(b)(1)(i) of this chapter.

(ii) *Culture media.* Use the medium described in § 440.80a(b)(1)(ii)(*b*) of this chapter for the seed layer and the base layer, except that the pH is adjusted to 5.8–6.0 after sterilization.

(iii) *Working standard.* Accurately weigh a suitable quantity of the chlortetracycline hydrochloride working standard and dissolve in 0.01 *N* HCl to make an appropriate stock solution. Keep in a glass-stoppered flask and store in the refrigerator for not more than 1 week.

(iv) *Preparation of sample.* Dissolve the sample to be tested in 0.01 *N* HCl to make an appropriate stock solution. Make the final dilution in 1 percent phosphate buffer, pH 4.5, to contain 0.10 microgram per milliliter (estimated).

(v) *Preparation of suspension.* The test organism is Bacillus cereus var. mycoides (ATCC 11778).[1] Maintain the test organism on slants of nutrient agar prepared as described in § 440.80a(b)(1)(ii)(*a*) of this chapter and transfer to fresh agar slants once a month, incubating overnight at 30° C. Streak several slants heavily with the test organism and incubate overnight at 30° C. Wash off the growth with approximately 5 milliliters of sterile distilled water onto the surface of a Roux bottle containing 300 milliliters of the nutrient agar. Spread the suspension over the entire agar surface with the aid of sterile glass beads. Incubate for 1 week at 30° C. Using 25 to 50 milliliters of sterile distilled water, wash the growth from the agar surface into a glass-stoppered Erlenmeyer flask. Heat-shock this suspension for 30 minutes at 65° C. Wash three times with sterile distilled water, centrifuging between each washing and then heat-shock for 30 minutes at 65° C. Resuspend in sterile distilled water and maintain in a refrigerator. This spore suspension may be kept for 1 month. Determine the percent of spore suspension that gives clear, sharp zones of adequate size after incubation with 0.10 microgram per milliliter of the test standard. Add the appropriate amount of the spore suspension to each 100 milliliters of the agar used for the seed layer, which has been melted and cooled to 48° C.

(vi) *Preparation of plates.* Add 21 milliliters of the agar prepared as in paragraph (b)(1)(ii) of this section to each Petri dish (20 millimeters x 100 millimeters). Distribute the agar evenly in the plates and allow it to harden. Use the plates the same day they are prepared. Add 4.0 milliliters of the inoculum as prepared in paragraph (b)(1)(v) of this section to each plate, tilting the plates back and forth to spread the inoculated agar evenly over the surface. Use porcelain covers glazed on the outside.

(vii) *Assay.* Place six cylinders on the inoculated agar surface so that they are at approximately 60° intervals on a 2.8 centimeter radius. Use three plates for each sample. Fill three cylinders on each plate with the 0.10 microgram per milliliter standard and three cylinders with the 0.10 microgram per milliliter (estimated) sample, alternating standard and sample. At the same time prepare a standard curve using concentrations of the standard of 0.064, 0.080, 0.10, 0.125, and 0.156 microgram per milliliter in phosphate buffer, pH 4.5. A total of 12 plates are used in the preparation of this standard curve, three plates for each solution, except the 0.10 microgram per milliliter solution. The latter concentration is used as the reference point and is included on each plate. On each of three plates fill three cylinders with the 0.10 microgram per milliliter standard and the other three cylinders with the concentration of the standard under test. Thus, there will be thirty-six 0.10 microgram determinations and nine determinations for each of the other points on the curve. Incubate the plates for 16 to 18 hours at 30° C. and measure the diameter of each circle of inhibition. Average the readings of the 0.10 microgram per milliliter concentration and the readings of the point tested for each set of three plates and average also all 36 readings of the 0.10 microgram per milliliter concentration. The average of the 36 readings of the 0.10 microgram per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 0.10 microgram per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 0.08 microgram per milliliter concentration the average of the 36 readings of the 0.10 microgram per milliliter concentration is 18.0 millimeters and the average of the 0.10 microgram per milliliter concentration of this set of three plates is 17.8 millimeters, the correction is 0.2 millimeter. If the average reading of the 0.08 microgram per milliliter concentration of those same three plates in 17.0 millimeters, the corrected value is then 17.2 millimeters. Plot these corrected values, including the average of the 0.10 microgram per milliliter concentration on one-cycle semilog paper, using the concentration in micrograms per milliliter as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Construct the best straight line through the points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

H = calculated zone diameter for the lowest concentration of the standard curve.

L = calculated zone diameter for the highest concentration of the standard curve.

c = average value of 36 zone diameters of the 0.1 microgram per milliliter standards.

a, b, d, e = corrected zone diameters for the 0.064, 0.080, 0.125, and 0.156 microgram per milliliter standard solutions, respectively.

Plot the value obtained for L and H and connect the points with a straight line. Use three plates for each sample. Fill three cylinders on each plate with the standard 0.10 microgram per milliliter solution and three cylinders on each plate with the 0.10 microgram per milliliter (estimated) sample, alternating standard and sample. Incubate all plates, including those containing the standard curve, at 30° C. overnight, and measure the diameter of each circle of inhibition. To estimate the potency of the sample, average the zone readings of the standard and the zone readings of the sample on the three plates used. If the sample gives a larger zone size than the average of the standard, add the difference between them to the 0.10 microgram per milliliter zone on the standard curve. If the average sample value is lower than the standard value, subtract the difference between them from the 0.10 microgram per milliliter value on the curve. From the standard curve, read the potencies corresponding to these corrected values of zone sizes.

(viii) *Turbidimetric assay.* In lieu of the plate-assay method described above, the sample may be assayed for potency by the following turbidimetric method:

(*a*) *Test culture and media.* The test organism is *Staphylococcus aureus* (ATCC 6538–P).[1] Prepare a Roux bottle suspension of this organism as described in § 440.80a(b)(1)(v) of this chapter. This suspension may be used for 2 weeks if stored in the refrigerator. Prepare the daily inoculum by adding 10 to 20 milliliters of the standardized suspension dilution to each liter of broth prepared as in § 440.80a(b)(1)(ii)(*c*) of this chapter.

(*b*) *Working standard.* Prepare a stock solution by dissolving an appropriate aliquot of the standard in sufficient 0.01 *N* HCl to give a concentration of 1,000 micrograms per milliliter. Keep in a glass-stoppered flask and store in the refrigerator for not more than 1 week. Prepare solutions for the daily standard curve by diluting an aliquot of the stock solution in 0.1 *M* phosphate buffer, pH 4.5, to the following concentrations: 0.036, 0.047, 0.060, 0.077, and 0.099 microgram per milliliter. Place 1 milliliter of each concentration in each of three replicate tubes 16 millimeters x 125 millimeters (outside dimension). To each tube, add 9 milliliters of inoculated broth described in paragraph (b)(1)(viii)(*a*) of this section and place immediately in a water bath at 37° C. for 3 to 4 hours. Remove the tubes and add 0.5 milliliter of a 12 percent solution of formaldehyde to each tube.

(*c*) *Preparation of sample.* Prepare an appropriate stock solution of the sample in 0.01 *N* HCl. Dilute further in pH 4.5 buffer to a final estimated concentration of 0.06 microgram per milliliter. Add 1 milliliter of this final concentration to each of three replicate tubes and proceed as described for the standard solution tubes in paragraph (b)(1)(viii)(*b*) of this section. After incubation and the addition of formaldehyde to all tubes, including standard and sample tubes, read the absorbance values in a suitable photoelectric colorimeter, using a wavelength of 530 mμ. Set the instrument at zero absorbance

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

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with clear, uninoculated broth prepared as described in § 440.80a(b)(1)(ii)(c).

(d) *Estimation of potency.* Plot the average absorbance valuer for each concentration of the standard on one-cycle semilogarithmic graph paper with absorbance values on the arithmetic scale and concentrations on the logarithmic scale. Construct the best straight line through the points either by inspection or by means of the equations described in § 436.515(a)(1)(v). Average the absorbance values for the sample and read the chlortetracycline concentration from the standard curve. Multiply the concentration by appropriate dilution factors to obtain the chlortetracycline content of the sample.

(ix) *Chlortetracycline content.* Its potency is satisfactory if the immediate containers contain 85 percent of the number of grams they are represented to contain.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Toxicity.* Proceed as directed in § 440.80a(b)(4) of this chapter, using as a test dose 0.5 milliliter of an aqueous solution containing 2 milligrams per milliliter.

(4) *Pyrogens.* Proceed as directed in § 440.80a(b)(3) of this chapter, using a test dose of 1.0 milliliter per kilogram of an aqueous solution containing 5 milligrams per milliliter.

(5) *Histamine.* Proceed as directed in § 444.70a(b)(5) of this chapter, using as a test dose 0.6 milliliter per kilogram of a solution containing 5 milligrams per milliliter prepared with the diluent recommended by the manufacturer in his labeling for the drug.

(6) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) or § 440.74a(b)(5) of this chapter.

(7) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(8) *Microscopical test for crystallinity.* Proceed as directed in § 440.80a(b)(5)(iii) of this chapter.

§ 446.15 Demeclocycline.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Demeclocycline is a hydrated compound of a kind of demeclocycline. It is so purified and dried that:

(i) Its potency is not less than 970 micrograms of demeclocycline hydrochloride equivalent per milligram on the anhydrous basis.

(ii) It passes the safety test.

(iii) Its moisture content is not less than 4.3 percent and not more than 6.7 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 4 and not more than 5.5.

(v) When calculated on the anhydrous basis, its absorptivity at 380 nanometers is 107.4±3.88 percent of that of the demeclocycline hydrochloride working standard similarly treated.

(vi) It is crystalline.

(vii) It passes the identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, moisture, pH, absorptivity, crystallinity, and identity.

(ii) Samples required: 10 packages, each containing approximately 250 milligrams.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of demeclocycline hydrochloride per milliliter (estimated). Further dilute an aliquot of the stock solution with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 0.100 microgram of demeclocycline hydrochloride per milliliter (estimated).

(2) *Safety.* Proceed as directed in § 436.33 of this chapter, preparing the test dose solution by dissolving 40 milligrams (as the anhydrous compound) in 2 milliliters of 0.1*N* HCl and diluting with the required amount of water.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Absorptivity.* Determine the percent absorptivity of the sample relative to that of the standard in the following manner: Dissolve an accurately weighed portion of approximately 40 milligrams of the sample in 2 milliliters of 0.1*N* HCl, dilute to exactly 250 milliliters with distilled water, and mix thoroughly. Transfer a 10-milliliter aliquot of this solution to a 100-milliliter volumetric flask. Add about 75 milliliters of distilled water and 5 milliliters of 5*N* NaOH, dilute to volume with distilled water, and mix thoroughly. Exactly 6 minutes after the addition of the NaOH, determine the absorbance of the solution at a wavelength of 380 nanometers, using a suitable spectrophotometer and distilled water as the blank. Treat a portion of the demeclocycline hydrochloride working standard in the same manner. Determine the percent relative absorptivity of the sample using the following calculation:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{weight of standard in milligrams} \times \text{potency of standard in micrograms per milligram} \times 10}{\text{Absorbance of standard} \times \text{weight of sample in milligrams} \times (100-m)}$$

where: m = percent moisture in the sample.

(6) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(7) *Identity.* Proceed as directed in § 446.16(b)(7). The value yielded by calculation ranges between 0.97 and 1.17.

§ 446.16 Demeclocycline hydrochloride.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Demeclocycline hydrochloride is the hydrochloride salt of a kind of demeclocycline. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram on the anhydrous basis.

(ii) It passes the safety test.

(iii) Its loss on drying is not more than 2 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2 and not more than 3.

(v) When calculated on the anhydrous basis, its absorptivity at 380 nanometers is 100±4.2 percent of that of the demeclocycline hydrochloride standard similarly treated.

(vi) It is crystalline.

(vii) It passes the identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, loss on drying, pH, absorptivity, crystallinity, and identity.

(ii) Samples required: 10 packages, each containing approximately 250 milligrams.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of demeclocycline hydrochloride per milliliter (estimated). Further dilute an aliquot of the stock solution with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 0.100 microgram of demeclocycline hydrochloride per milliliter (estimated).

(2) *Safety.* Proceed as directed in § 436.33 of this chapter.

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Absorptivity.* Determine the percent absorptivity of the sample relative to that of the standard in the following manner: Dissolve an accurately weighed portion of approximately 40 milligrams of the sample in 2 milliliters of 0.1*N* HCl, dilute to exactly 250 milliliters with distilled water, and mix thoroughly. Transfer a 10 milliliter aliquot of this solution to a 100-milliliter volumetric flask. Add about 75 milliliters of distilled water and 5 milliliters of 5*N* NaOH, dilute to volume with distilled water, and mix thoroughly. Exactly 6 minutes after the addition of the NaOH, determine the

absorbance of the solution at a wavelength of 380 nanometers, using a suitable spectrophotometer and distilled water as the blank. Treat a portion of the working standard in the same manner. Determine the percent relative absorptivity of the sample using the following calculation:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{weight of standard in milligrams} \times \text{potency of standard in micrograms per milligram} \times 10}{\text{Absorbance of standard} \times \text{weight of sample in milligrams} \times (100-m)}$$

where: m = percent moisture in the sample.

(6) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(7) *Identity.* Accurately weigh 40 milligrams of the sample and place into a 200-milliliter volumetric flask. Add 100 milliliters of 0.1*N* HCl and place on a shaker until the sample is dissolved. Dilute to volume with 0.1*N* HCl and mix well. Transfer a 5-milliliter aliquot of the solution to each of two 50-milliliter volumetric flasks. To one flask add 10 milliliters of 6*N* HCl and to the other add 10 milliliters of 3*N* HCl. Place the acid-treated flasks into a boiling water bath for 20 minutes. Remove the flasks and place in a cold water bath. When cool, dilute to volume with water and mix well. Treat a portion of the standard in the same manner. Using a suitable spectrophotometer, place the 6*N* HCl-treated sample into the reference cell and read against the 3*N* HCl-treated sample at a wavelength of 368 nanometers. Reverse the order of the cells in the cell holder and read at a wavelength of 430 nanometers.

$$\frac{(A_{368}+A_{430}\text{ sample})\text{ (milligrams of standard per milliliter) (100)}}{(A_{368}+A_{430}\text{ standard})\text{ (milligrams of sample per milliliter) (100-}m\text{)}} = 0.9 \text{ to } 1.1$$

where: m = percent moisture in the sample.

§ 446.20 Doxycycline hyclate.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Doxycycline hyclate is a hydrochloride hemiethanolate hemihydrate salt of 6-deoxy-oxytetracycline. It is so purified and dried that:

(i) Its potency is not less than 800 nor more than 920 micrograms of doxycycline per milligram on an "as is" basis.

(ii) It passes the safety test.

(iii) Its moisture content is not less than 1.4 nor more than 2.75 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.0 nor more than 3.0.

(v) It contains not less than 82 nor more than 90 percent doxycycline on an "as is" basis.

(vi) It gives a positive identity test for doxycycline hyclate.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, moisture, pH, doxycycline content, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of doxycycline per milliliter (estimated). Further dilute with 0.1*M* potassium phosphate buffer pH 4.5 (solution 4), to the reference concentration of 0.100 microgram of doxycycline per milliliter (estimated).

(2) *Safety.* Proceed as directed in § 436.33 of this chapter.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using an aqueous solution containing the equivalent of 10 milligrams of doxycycline per milliliter.

(5) *Doxycycline content—*(i) *Equipment—*(a) *Sheet (chromatographic).* Whatman No. 4 filter paper for chromatography, 15 x 57 centimeters.

(b) *Chamber (chromatographic).* Square glass chromatography jar, 30 x 30 x 60 centimeters, equipped with 25-centimeter troughs for descending chromatography.

(ii) *Preparation of solutions—*(a) *0.05N Methanolic hydrochloric acid.* Dilute 4.2 milliliters of concentrated hydrochloric acid to 1 liter with methanol.

(b) *pH 4.2 buffer.* Mix 5.86 volumes of 0.1*M* citric acid with 4.14 volumes of 0.2*M* disodium phosphate.

(c) *Chromatographic system.* Mix toluene, pyridine, and pH 4.2 buffer in volumetric proportions of 20:3:10, respectively. Allow the phases to separate. Place the upper phase in the troughs near the top of the chamber. Place the lower phase in the bottom of the chamber. Saturate the atmosphere of the tightly sealed chamber for 24 hours before use by placing white blotters on two opposite sides of the chamber so that their ends are immersed in the lower phase in the bottom of the chamber. Replace the solvent in troughs before the chromatograms are to be developed.

(iii) *Preparation of the doxycycline standard solution.* Accurately weigh about 50 milligrams of the doxycycline working standard into a 5-milliliter volumetric flask and bring to volume with 0.05*N* methanolic hydrochloric acid. Store in the refrigerator and use within 7 days.

(iv) *Preparation of sample.* Accurately weigh about 50 milligrams of the sample into a 5-milliliter volumetric flask and bring to volume with 0.05*N* methanolic hydrochloric acid.

(v) *Preparation of the chromatogram.* Dip the chromatographic sheets into pH 4.2 buffer and lightly blot each sheet between clean nonfluorescing, white blotters. Use separate sheets for the doxycycline standard solution, for each doxycycline sample solution, and for blanks without standard or sample application. Care must be taken so that the moist sheets do not become too dry; a period of 5 to 10 minutes between impregnating the paper and placing it in the chromatographic chamber is usually satisfactory. Evenly apply a 0.100-milliliter aliquot of a doxycycline solution to the origin line of a sheet as a 14-centimeter-long streak. Place the sheets in the chamber and develop them in a descending manner for 2 hours. The doxycycline band should move approximately 12.5 centimeters from the origin line. Remove the sheets from the chamber and air-dry for about 10 minutes.

(vi) *Processing the chromatogram.* Examine each sheet under 366-nanometer ultraviolet light. Outline the fluorescent bands with a pencil. The main marked area should be approximately 10 x 15 centimeters in size. Outline areas on the blank sheet approximately equal in size and in the same locations as those outlined on the standard sheet. Exposure of the sheets to ammonia or other alkaline vapors must be avoided. Cut the marked areas from the sheets and then cut them into approximately 2-centimeter squares. For each sheet, place the squares from each of the following areas into separate 125-milliliter Erlenmeyer flasks: The main doxycycline band of the sample, the main doxycycline band of the standard, all the other bands of the standard, the area of the blank sheet corresponding to the main band of the standard, the other area of the blank sheet corresponding to the other bands of the standard. The time between removing the sheets from the chamber and placing the squares into the Erlenmeyer flasks should be minimal, since excessive drying of the paper can lead to erratic elutions.

(vii) *Elution.* To each flask add 50 milliliters of 0.05*N* methanolic hydrochloric acid and agitate on a reciprocating shaker for 1 hour. Decant the contents of each flask into another flask by pouring through a small funnel fitted with a glass wool plug.

(viii) *Doxycycline standard solution for direct measurement of absorbance.* Pipette a 0.100-milliliter aliquot of the doxycycline standard solution into each of three 125-milliliter Erlenmeyer flasks. Add 50 milliliters of 0.05*N* methanolic hydrochloric acid to each of these flasks.

(ix) *Absorbance measurement.* Using a suitable spectrophotometer and 0.05*N* methanolic hydrochloric acid as the reference solvent, determine the absorbance of each eluate and of each doxycycline standard solution at the absorption maximum at about 349 nanometers.

(x) *Calculation of percent doxycycline in samples.* Calculate as follows:

$$\text{Percent doxycycline} = \frac{(A_s - A_b)(W_s)}{(A_c - A_b)(W_c)} \times \text{Doxycycline content of the working standard, where:}$$

A_s=Absorbance of the eluate from the main doxycycline band of the sample sheet.
A_c=Absorbance of the eluate from the main doxycycline band on the standard sheet.
A_b=Absorbance of the eluate from the area of the blank sheet corresponding to the area of the doxycycline band of the standard sheet.
W_s=Weight in milligrams of sample.
W_c=Weight in milligrams of doxycycline working standard.

(xi) *Recovery of the doxycycline standard from the chromatogram.* As follows:

$$\text{Percent recovery} = \frac{As - Ab}{Ad} \times \frac{100}{F},$$

where:

A_D=Absorbance of the doxycycline standard solution described in paragraph (b)(5)(viii) of this section.
F=The fractional purity of doxycycline standard solution described in paragraph (b)(5)(xii) of this section.

If the recovery of the doxycycline standard from the chromatogram is less than 95 percent, repeat the chromatogram.

(xii) *Determination of the fractional purity of the doxycycline working standard.* Determine F by means of the following equation:

$$F = 1 - \frac{(Ac - Acb)}{(Ac - Acb + As - Ab)},$$

where:

A_c=Absorbance of the eluate from sections of the standard chromatogram containing nondoxycycline 349 nanometers-absorbing contaminants.
A_{cb}=Absorbance of the eluates from the sections of the blank sheets corresponding to those sections of the nondoxycycline-absorbing contaminants of the standard sheets.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using the 0.25 potassium bromide mixture described in paragraph (b)(1) of that section.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

§ 446.20a Sterile doxycycline hyclate.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Sterile doxycycline hyclate is a hydrochloride hemiethanolate hemihydrate salt of 6-deoxy-oxytetracycline. It is so purified and dried that:

(i) Its potency is not less than 800 nor more than 920 micrograms of doxycycline per milligram on an "as is" basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It passes the safety test.

(v) It contains no histamine nor histamine-like substances.

(vi) Its moisture content is not less than 1.4 nor more than 2.75 percent.

(vii) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.0 nor more than 3.0.

(viii) It contains not less than 82 nor more than 90 percent doxycycline on an "as is" basis.

(ix) It gives a positive identity test for doxycycline hyclate.

(x) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, safety, histamine, moisture, pH, doxycycline content, identity, and crystallinity.

(ii) Samples required:

(*a*) For all tests except sterility: 12 packages, each containing approximately 300 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of doxycycline per milliliter (estimated). Further dilute with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 0.100 microgram of doxycycline per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this subchapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this subchapter, using a solution containing 7.5 milligrams of doxycycline per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this subchapter.

(5) *Histamine.* Proceed as directed in § 436.35 of this subchapter.

(6) *Moisture.* Proceed as directed in § 436.201 of this subchapter.

(7) *pH.* Proceed as directed in § 436.202 of this subchapter, using an aqueous solution containing the equivalent of 10 milligrams of doxycycline per milliliter.

(8) *Doxycycline content.* Proceed as directed in § 446.20(b)(5).

(9) *Identity.* Proceed as directed in § 436.211 of this subchapter, using the 0.25 potassium bromide mixture described in paragraph (b)(1) of that section.

(10) *Crystallinity.* Proceed as directed in § 436.203(a) of this subchapter.

§ 446.21 Doxycycline monohydrate.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Doxycycline monohydrate is a hydrated compound of doxycycline. It is so purified and dried that:

(i) Its potency is not less than 880 micrograms nor more than 980 micrograms of doxycycline per milligram on an "as is" basis.

(ii) It passes the safety test.

(iii) Its moisture content is not less than 3.6 percent nor more than 4.6 percent.

(iv) Its pH in an aqueous suspension containing the equivalent of 10 milligrams of doxycycline per milliliter is not less than 5.0 nor more than 6.5.

(v) It contains not less than 90 percent nor more than 98 percent doxycycline on an "as is" basis.

(vi) It gives a positive identity test for doxycycline monohydrate.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, moisture, pH, doxycycline content, identity, and crystallinity.

(ii) Samples of the batch: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of doxycycline per milliliter (estimated). Further dilute with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 0.100 microgram of doxycycline per milliliter (estimated).

(2) *Safety.* Proceed as directed in § 436.33 of this chapter, except evaluate the results after observing the mice for 5 days and prepare the sample as follows: Transfer 1 to 2 grams of the sample, accurately weighed, to a mortar. Add 1 drop of polysorbate 80 and while grinding with a pestle, slowly add sufficient sterile, distilled water to make a suspension containing 100 milligrams of doxycycline per milliliter.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous suspension containing the equivalent of 10 milligrams of doxycycline per milliliter.

(5) *Doxycycline content.* Proceed as directed in § 446.20(b)(5).

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using the 0.25 potassium bromide mixture described in paragraph (b)(1) of that section.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

§ 446.50 Methacycline hydrochloride.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Methacycline hydrochloride is the hydrochloride salt of the 6-methylene homolog of oxytetracycline or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 832 micrograms of methacycline per milligram on an "as is" basis.

(ii) It passes the safety test.

(iii) Its moisture content is not more than 2 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.0 nor more than 3.0.

(v) Its absorptivity at the absorption maximum of 345 nanometers is 92.4±4 percent of the methacycline standard similarly treated.

(vi) It gives a positive result to the identity test for methacycline hydrochloride.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, moisture, pH, absorptivity, identity, and crystallinity.

(ii) Samples of the batch: 10 packages, each containing 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.01*N* methanolic hydrochloric acid (solution 13) to obtain a concentration of 1 milligram of methacycline per milliliter. Further dilute with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 0.06 microgram of methacycline per milliliter (estimated).

(2) *Safety.* Proceed as directed in § 436.33 of this chapter.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams of methacycline per milliliter.

(5) *Absorptivity.* Determine the absorbance of the sample and standard solutions in the following manner: Dissolve approximately 50 milligrams each of the sample and standard in 100 milliliters of 0.01*N* methanolic hydrochloric acid. Transfer a 10-milliliter aliquot to a 250-milliliter volumetric flask and dilute to volume with 0.01*N* methanolic hydrochloric acid. Using a suitable spectrophotometer and 0.01*N* methanolic hydrochloric acid as the blank, scan the absorption spectrum between the wavelengths of 250 and 400 nanometers. Determine the absorbance of each solution at the maxima, ca. 345 nanometers. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \frac{\text{weight in milligrams of standard}}{\text{potency of standard in micrograms per milligram}}}{\text{Absorbance of standard} \times \text{weight in milligrams of sample} \times 10}$$

(6) *Identity.* The absorption spectrum between the wavelength of 250 and 400 nanometers, determined as directed in paragraph (b)(5) of this section, compares qualitatively with that of the methacycline standard.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

§ 446.60 Minocycline hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Minocycline hydrochloride is the hydrochloride salt of 7-dimethylamino-6-deoxy-6-demethyltetracycline. It is so purified and dried that:

(i) Its potency is not less than 785 micrograms per milligram on an "as is" basis.

(ii) It passes the safety test.

(iii) Its moisture content is not less than 4.3 percent and not more than 8.0 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams of minocycline per milliliter is not less than 3.5 and not more than 4.5.

(v) It contains not less than 78.5 percent and not more than 88.5 percent minocycline on an "as is" basis.

(vi) It gives a positive identity test for minocycline hydrochloride.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, moisture, pH, minocycline content, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to give a stock solution of convenient concentration containing not less than 150 micrograms of minocycline per milliliter (estimated). Further dilute the stock solution with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 0.100 microgram of minocycline per milliliter (estimated).

(2) *Safety.* Proceed as directed in § 436.33 of this chapter.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams of minocycline per milliliter.

(5) *Minocycline content*—(i) *Apparatus and reagents.* (a) Glass chromatographic column (1.3 centimeters ID x 35 centimeters long).

(b) Diatomaceous earth, acid-washed (Celite 545 or equivalent). Slurry 200 grams of diatomaceous earth with 750 milliliters of 6*N* hydrochloric acid for 10 to 15 minutes in a glass vessel. Filter and wash the diatomaceous earth with water until the pH is neutral. Slurry the neutral diatomaceous earth in 500 milliliters of methyl alcoholethyl acetate mixture (1:1 by volume) for 10 to 15 minutes. Filter and vacuum dry the diatomaceous earth at 60° C.

(c) EDTA solution: 0.1*M* ethylenediaminetetraacetic acid disodium salt adjusted to pH 6.0 with ammonium hydroxide.

(d) 20 percent polyethylene glycol 400 in glycerine: To 80 milliliters of glycerine, add sufficient polyethylene glycol 400 to make a volume of 100 milliliters.

(e) Reagent A (5 percent polyethylene glycol 400—glycerine mixture in EDTA solution): To 95 milliliters of EDTA solution, add sufficient 20 percent polyethylene glycol 400 in glycerine to make a volume of 100 milliliters, and shake well.

(f) Acid-alcohol reagent: Transfer 2 milliliters of hydrochloric acid to a 100-milliliter volumetric flask, dilute to mark with absolute ethyl alcohol, and mix.

(g) Column support: Place 200 grams of acid-washed diatomaceous earth into a 2-liter wide-mouth bottle with a screw top and add 100 milliliters of Reagent A. Seal the bottle and shake vigorously. Roll for 2 hours on a rolling mill until the diatomaceous earth is uniformly moistened.

(ii) *Column preparation.* Place a small circle of filter paper (Whatman No. 541 or equivalent, about 1.2 centimeters in diameter) as a plug in the bottom of the column. Firmly pack the column with 8 grams ±0.1 gram of column support in three increments. Between increments, tap the column lightly on the bench top. Press each portion evenly and firmly with a glass tamping rod. Each portion should be flat across the entire surface with no feathery edges creeping up the column walls.

(iii) *Sample preparation.* Weigh accurately a portion of sample equivalent to 20 milligrams of minocycline, transfer to a 25-milliliter volumetric flask using 4 milliliters of water, and swirl to dissolve. Dilute to mark with Reagent A and shake well to insure complete solution. Pipet 1.0 milliliter of the sample solution into a 50-milliliter beaker containing 2 grams of acid-washed diatomaceous earth and stir well. Transfer the sample-diatomaceous earth mixture to the column as completely as possible and tamp firmly. To rinse the 50-milliliter sample beaker, add 0.5 gram of column support and transfer to the top of the column and tamp firmly.

(iv) *Column elution.* Place the column in a suitable support and place a 25-milliliter graduate under the column. Add 25 milliliters of 15 percent chloroform in cyclohexane to the column. When the level of this solvent reaches the charge at the top of the column, add 50 milliliters of 30 percent chloroform in cyclohexane. Collect 22 milliliters of the first eluate in the 25-milliliter graduate and discard. Replace the 25-milliliter graduate with a 50-milliliter volumetric flask. Collect the second eluate in this 50-milliliter volumetric flask until the level of the solvent reaches the charge.

Replace the 50-milliliter volumetric flask with a 10-milliliter graduate and add 30 milliliters of chloroform to the column. When the volume of the third eluate in the graduate reaches 8 milliliters, replace the 10-milliliter graduate with a 25-milliliter glass-stoppered graduate and continue collecting the fourth eluate until no more solvent drips from the column.

(v) *Determination of minocycline content.* Transfer the 8 milliliters of third eluate contained in the 10-milliliter graduate to the 50-milliliter volumetric flask containing the second eluate, and mix. Add 2 milliliters of absolute ethyl alcohol followed by 2 milliliters of acid-alcohol reagent, dilute to mark with chloroform and mix. Using a suitable spectrophotometer, 1.0 centimeter cells, and chloroform as the reference solvent, determine the absorbance of this solution at 358 nanometers within 10 minutes after preparation of the solution.

(vi) *Determination of minocycline related compounds content.* To the last 25-milliliter glass-stoppered graduate, add 2 milliliters of acid-alcohol reagent, dilute to 25 milliliters with chloroform, and mix. Using a suitable spectrophotometer, 1.0 centimeter cells, and chloroform as the reference solvent, determine the absorbance of this solution at 358 nanometers within 10 minutes after preparation of the solution.

(vii) *Preparation of standard.* Weigh accurately an amount of minocycline hydrochloride working standard equivalent to 20 milligrams of minocycline, transfer to a 100-milliliter volumetric flask, and dissolve by adding 5 milliliters of methyl alcohol. Dilute to mark with chloroform and mix. Pipet 4 milliliters into a 50-milliliter volumetric flask, add 40 milliliters of 30 percent chloroform in cyclohexane, and swirl to mix. Add 2 milliliters of absolute ethyl alcohol followed by 2 milliliters of acid-alcohol reagent, dilute to mark with chloroform, and mix. Using a suitable spectrophotometer, 1.0 centimeter cells, and chloroform as the reference solvent, determine the absorbance of this solution at 358 nanometers within 10 minutes after preparation of the solution.

(viii) *Calculations for minocycline content*

$$\text{Percent minocycline "as is"} = \frac{A_s \times W_s \times \text{potency of the standard in micrograms of minocycline per milligram}}{A_r \times W_r \times 10},$$

where:

A_s = Absorbance of the combined second and third eluates of the sample.
A_r = Absorbance of the standard.
W_s = Weight in milligrams of sample.
W_r = Weight in milligrams of the standard.

$$\text{Percent minocycline related compounds} = \frac{A_r \times W_r \times \text{potency of the standard in micrograms of minocycline per milligram}}{A_s \times W_s \times 20},$$

Where:

A_r = Absorbance of the fourth eluate of the sample (minocycline related compounds).

(ix) *Column recovery—(a) Procedure.* Weigh accurately about 20 milligrams of sample and proceed as directed in paragraph (b)(5)(vii) of this section.

(b) *Calculations.*

$$\text{Percent total minocycline of sample} = \frac{A_d \times W_d \times \text{potency of the standard in micrograms of minocycline per milligram}}{A_s \times W_d \times 10},$$

where:

A_d = Absorbance by direct measurement of unchromatographed sample solution.
W_d = Weight of sample for direct absorbance measurement.

$$\text{Percent column recovery} = \frac{\text{percent minocycline of sample} + \text{percent minocycline related compounds of sample} \times 100}{\text{percent total minocyclines of sample}},$$

To be a valid assay, the percent column recovery should be 100±2 percent.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using a 0.5 percent potassium bromide disc prepared as described in paragraph (b)(1) of that section.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

§ 446.65a Sterile oxytetracycline.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Oxytetracycline is the hydrated or anhydrous crystalline compound of a kind of oxytetracycline, or a mixture of two or more such compounds. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms of oxytetracycline per milligram on an anhydrous basis.

(ii) It is sterile.

(iii) It is nontoxic.

(iv) It is nonpyrogenic.

(v) It contains no histamine nor histamine-like substance.

(vi) Its moisture content is not more than 9 percent.

(vii) Its pH in an aqueous suspension containing 10 milligrams per milliliter is not less than 4.5 and not more than 7.0.

(viii) When calculated on the anhydrous basis, its absorptivity at 353 millimicrons is 100±4 percent of that of the oxytetracycline standard similarly treated and corrected for potency.

(ix) It gives a positive result to an identity test for oxytetracycline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, toxicity, pyrogens, histamine, moisture, pH, absorptivity, crystallinity, and identity.

(ii) Samples required:

(a) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(b) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay—(1) Potency—(i) Plate assay—(a) Cylinders (cups).* Use cylinders described in § 440.80a(b)(1)(i) of this chapter.

(b) *Culture media.* Use ingredients that conform to the standards prescribed by the U.S.P. or N.F.

(1) Make nutrient agar for maintaining the test organism as follows:

Peptone	6.0 gm.
Pancreatic digest of casein	4.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Dextrose	1.0 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 6.5 to 6.6 after sterilization.

(2) Make nutrient agar for the seed layer and for the base layer as follows:

Peptone	6.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 5.8 to 6.0 after sterilization.

In lieu of preparing the media from the individual ingredients specified in this subsection, the media may be made from a dehydrated mixture which, when reconstituted with distilled water, has the same composition as such media. Minor modifications of the individual ingredients specified are permissible if the resulting medium possesses growth-promoting properties at least equal to the medium described.

(c) *Working standard.* Accurately weigh a suitable quantity of the oxytetracycline working standard and dissolve in .01*N* hydrochloric acid to make an appropriate stock solution. Keep in a glass-stoppered flask and store under refrigeration. Discard solution after 1 week.

(d) *Preparation of sample.* Dissolve the sample to be tested in 0.1*N* hydrochloric acid to make an appropriate stock solution. Make the final dilution in 0.1*M* potassium phosphate buffer, pH 4.5, to contain 1.0 microgram per milliliter (estimated).

(e) *Preparation of suspension.* The test organism is *Bacillus cereus* var. *mycoides* (ATCC 11778)[1], which is maintained on slants of agar described in paragraph (b)(1)(i)(*b*)(*1*) of this section. Wash the organism from the agar slant with 3 milliliters of sterile U.S.P. saline T.S. onto a large agar surface such as that provided by a Roux bottle containing 300 milliliters of the agar described in paragraph (b)(1)(i)(*b*)(*1*) of this section. Spread the suspension of organism over the entire agar surface with the aid of sterile glass beads. Incubate for 1 week at 37° C. Using 50 milliliters of sterile U.S.P. saline T.S., wash the growth from the agar surface into a glass-stoppered Erlenmeyer flask. Heat-shock this suspension for 30 minutes at 70° C. Wash three times with sterile U.S.P. saline T.S., centrifuging between each washing, and then heat-shock for 30 minutes at 70° C. Resuspend in 50 milliliters of sterile U.S.P. saline T.S. and maintain in a refrigerator. This spore suspension may be kept for 1 month. Run test plates to determine the quantity of spore suspension (usually 0.1 milliliter) that should be added to each 100 milliliters of agar to give clear, sharp zones of inhibition of appropriate size.

(f) *Preparation of plates.* Add 21 milliliters of the agar prepared as described in paragraph (b)(1)(i)(*b*)(*2*) of this section to each Petri dish (20 millimeters x 100 millimeters). Distribute the agar evenly in the plates, and allow it to harden. Use the plates the same day they are prepared. Melt a sufficient amount of the agar described in paragraph (b)(1)(i)(*b*)(*2*) of this section, cool to 48° C., add the proper amount of the test organism as described in paragraph (b)(1)(i)(*d*) of this section, and mix thoroughly. Add 4 milliliters of this inoculated agar to each Petri dish. Distribute the agar evenly in the plates, cover with porcelain covers glazed on the outside, and allow to harden. Place 6 cylinders on the agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius.

(g) *Standard curve.* Prepare the daily standard curve by further diluting the stock working standard solution in 0.1*M* potassium phosphate buffer, pH 4.5, to obtain concentrations of 0.64, 0.80, 1.0, 1.25, and 1.56 micrograms per milliliter. Use three plates for the determination of each point on the curve, except the 1.0 microgram per milliliter concentration, a total of 12 plates. On each of three plates fill three cylinders with the 1.0 micrograms per milliliter standard, and the other three cylinders with the concentration under test. Thus, there will be thirty-six 1-microgram determinations and 9 determinations for each of the other points on the curve. After incubation, read the diameters of the circles of inhibition of the plates. Average the readings of the 1.0 microgram per milliliter concentration and the readings of the point tested for each set of three plates and average also all 36 readings of the 1.0 microgram per milliliter concentration. The average of the 36 readings of the 1.0 microgram per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 1.0 microgram per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 0.8 microgram per milliliter concentration, the average of the 36 readings of the 1.0 microgram concentration were 20.0 millimeters, and the average of the 1.0 microgram concentration of this set of three plates were 19.8 millimeters, the correction would be +0.2 millimeter. If the average reading of the 0.8-microgram concentration of these same three plates were 19.0 millimeters, the corrected value would be 19.2 millimeters. Plot these corrected values, including the average of the 1.0 microgram per milliliter concentration, on 2-cycle semilog paper, using the concentration in micrograms per milliliter as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-s}{5},$$

$$H=\frac{3s+2d+c-a}{5},$$

where:

L = Calculated zone diameter for the lowest concentration of the standard curve;

H = Calculated zone diameter for the highest concentration of the standard curve;

c = Average zone diameter of 36 readings of the 1.0 microgram per milliliter standard;

a, b, d, s = Corrected average values for the 0.64, 0.80, 1.25, and 1.56 micrograms per milliliter standard solutions, respectively.

Plot the values obtained for L and H and connect with a straight line.

(h) *Assay.* Use three plates for each sample. Fill three cylinders on each plate with the standard 1.0 microgram per milliliter solution and three cylinders with the 1.0 microgram per milliliter (estimated) sample, alternating standard and sample. Incubate all plates, including those containing the standard curve, at 30° C. overnight, and measure the diameter of each circle of inhibition. To estimate the potency of the sample, average the zone readings of the standard and the zone readings of the sample on the three plates used. If the sample gives a larger zone size than the average of the standard, add the difference between them to the 1.0 microgram per milliliter zone on the standard curve. If the average sample value is lower than the standard value, subtract the difference between them from the 1.0 microgram per milliliter value on the curve. From the standard curve, read the potencies corresponding to these corrected values of zone sizes.

(ii) *Turbidimetric assay.* In lieu of the plate assay method described in paragraph (b)(1)(i)(*h*) of this section, the sample may be assayed for potency by the following turbidimetric method:

(a) *Nutrient broth:* Make nutrient broth for preparing an inoculum of the test organism as follows:

Peptone	5.0 gm.
Yeast extract	1.5 gm.
Beef extract	1.5 gm.
Sodium chloride	3.5 gm.
Dextrose	1.0 gm.
Dipotassium phosphate	3.68 gm.
Potassium dihydrogen phosphate	1.32 gm.
Distilled water, q.s.	1,000.0 ml.

pH 7.0 after sterilization.

In lieu of preparing the medium from the individual ingredients specified in this paragraph (b)(1)(ii)(*a*), the medium may be made from a dehydrated mixture which, when reconstituted with distilled water, has the same composition as such medium. Minor modifications of the individual ingredients specified are permissible if the resulting medium possesses growth-promoting properties at least equal to the medium described.

(b) *Working standard.* Accurately weigh a suitable quantity of the oxytetracycline working standard and dissolve in 0.1*N* hydrochloric acid to make an appropriate stock solution. Keep in a glass-stoppered flask and store under refrigeration. Discard solution after 1 week.

(c) *Preparation of sample.* Dissolve an appropriate quantity of the sample in a volume of 0.1*N* hydrochloric acid to obtain a convenient stock solution. Make other dilutions volumetrically in 0.1*M* potassium phosphate buffer, pH 4.5, to a final concentration of 0.24 microgram of oxytetracycline base per milliliter (estimated).

(d) *Preparation of test organism.* The test organism is *Staphylococcus aureus* (ATCC 6538P),[1] which is maintained on agar described in paragraph (b)(1)(i)(*b*)(*1*) of this section. From a stock slant, inoculate a Roux bottle containing the same agar and incubate for 24 hours at 32° C. to 35° C. Wash the growth from the agar surface with 50 milliliters of sterile U.S.P. saline T.S. Standardize the resulting bulk suspension so that a 1:20 dilution in U.S.P. saline T.S. will give 25 percent light transmission, using a suitable photoelectric colorimeter with a 580-millimicron filter and a 13-millimeter diameter test tube as an absorption cell. For the daily inoculum, use approximately 3.0 milliliters of the bulk suspension for each 1,000 milliliters of nutrient broth needed in the assay.

(e) *Procedure.* Prepare solutions for the daily standard curve by diluting an aliquot of the stock working standard solution prepared as described in paragraph (b)(1)(ii)(*b*) of this section in 0.1*M* potassium phosphate buffer, pH 4.5, to the following concentrations: 0.146, 0.187, 0.240, 0.308, and 0.395 microgram per milliliter. Place 1.0 milliliter of each concentration of the standard curve and of the sample solution prepared as described in paragraph (b)(1)

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

(ii) (c) of this section in each of three replicate test tubes (16 millimeters x 125 millimeters). To each tube add 9 milliliters of the inoculated broth described in paragraph (b) (1) (ii) (d) of this section, and place immediately in a water bath at 37° C. for 3 to 4 hours. Remove the tubes and add 0.5 milliliter of a 12 percent solution of formaldehyde to each tube. Read the absorbance values of each tube in a suitable photoelectric colorimeter, using a wavelength of 530 millimicrons. Set the instrument at zero absorbance with clear, uninoculated broth prepared as described in paragraph (b) (1) (ii) (a) of this section.

(f) *Estimation of potency.* Plot the average absorbance values for each concentration of the standard on semilogarithmic graph paper with absorbance values on the arithmetic scale and concentrations on the logarithmic scale. Construct the best straight line through the points either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

L = Calculated absorbance value for the lowest concentration of the standard curve;

H = Calculated absorbance value for the highest concentration of the standard curve;

a, b, c, d, e = Average absorbance values for each concentration of the standard curve.

Plot the values obtained for L and H and connect the points with a straight line. Average the absorbance value for the sample and read the oxytetracycline concentration from the standard curve. Multiply the concentration by appropriate dilution factors to obtain the oxytetracycline content of the sample.

(iii) *Chemical assay.* In lieu of the biological assay methods described in this paragraph, the sample may be assayed for potency by the following chemical assay method:

(a) *Reagents.* (1) 1*N* hydrochloric acid.

(2) 0.01*N* hydrochloric acid.

(3) Ferric chloride stock solution.

Quickly weigh (very hygroscopic) 5.0 grams of $FeCl_3 \cdot 6H_2O$ into a 100-milliliter beaker. Add approximately 10 milliliters of 1*N* hydrochloric acid, and stir to dissolve. Quantitatively transfer to a 50-milliliter, glass-stoppered, amber volumetric flask, and make up to volume with water.

(4) Ferric chloride working reagent.

Pipette 10.0 milliliters of ferric chloride stock solution to a 2-liter volumetric flask, add 20.0 milliliters 1*N* hydrochloric acid and bring to volume with water. Check the pH; it should be between 2.0 and 2.1.

(b) *Standard solution.* Accurately weigh approximately 50 milligrams of the oxytetracycline working standard and dissolve with 25 milliliters of 0.1*N* hydrochloric acid. Quantitatively transfer to a 250-milliliter volumetric flask and dilute to volume with distilled water. Keep in a glass-stoppered flask and store under refrigeration. Discard solution after 1 week.

(c) *Sample solution.* Prepare the sample solution as directed in paragraph (b) (1) (i) (b) of this section.

(d) *Procedure.* Pipette exactly 10.0 milliliters of the standard solution and of the sample solution into separate test tubes. To each tube add exactly 10.0 milliliters of ferric chloride working reagent, mix and allow to stand 15 minutes. Determine the absorbance of each solution at 490 millimicrons in a suitable spectrophotometer against a blank prepared from 10 milliliters of 0.01*N* hydrochloric acid and 10 milliliters of ferric chloride working reagent.

(e) *Estimation of potency.* Calculate the potency as follows:

$$\text{Micrograms of oxytetracycline per milligram} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{Milligrams of standard}}{\text{Milligrams of sample}} \times \text{Potency of standard in micrograms per milligram} \times \frac{100}{(100-m)}$$

where m = percent moisture in the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e) (1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Toxicity.* Proceed as directed in § 440.80a(b) (4) of this chapter, using as a test dose 0.5 milliliter of an aqueous solution containing 2.0 milligrams per milliliter prepared by dissolving 40 milligrams in 2.0 milliliters of 0.1*N* hydrochloric acid and diluting with the required amount of sterile distilled water.

(4) *Pyrogens.* Proceed as directed in § 440.80a(b) (3) of this chapter, using as a test dose 1.0 milliliter per kilogram of a solution containing 5.0 milligrams of oxytetracycline per milliliter, prepared by dissolving 40 milligrams in 2.0 milliliters of 0.1*N* hydrochloric acid and diluting with the required amount of sterile, pyrogen-free distilled water.

(5) *Histamine.* Proceed as directed in § 444.70a(b) (5) of this chapter, using as a test dose 0.6 milliliter per kilogram of a sterile distilled water solution containing 5 milligrams per milliliter, prepared by dissolving 40 milligrams in 2.0 milliliters of 0.1*N* hydrochloric acid and diluting with the required amount of sterile distilled water.

(6) *Moisture.* Proceed as directed in § 440.74a(b) (5) of this chapter.

(7) *pH.* Proceed as directed in § 440.80a (b) (5) (ii) of this chapter, using an aqueous suspension containing 10 milligrams per milliliter.

(8) *Absorptivity.* Determine the absorbance of the sample and standard solutions in the following manner: Dissolve approximately 50 milligrams each of the sample and standard in 250 milliliters of 0.1*N* hydrochloric acid. Transfer a 10-milliliter aliquot to a 100-milliliter volumetric flask, and dilute to volume with 0.1*N* hydrochloric acid. Using a suitable spectrophotometer and 0.1*N* hydrochloric acid as the blank, determine the absorbance of each solution at 353 millimicrons. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{milligrams standard} \times \text{potency of standard in micrograms per milligram} \times 10}{\text{Absorbance of standard} \times \text{milligrams sample} \times (100-m)}$$

where m = percent moisture in the sample.

(9) *Crystallinity.* Proceed as directed in § 440.80a(b) (5) (iii) of this chapter.

(10) *Identity.* To about 1 milligram of sample, add 2 milliliters of sulfuric acid; a light-red color is produced when oxytetracycline is present.

§ 446.66 Oxytetracycline calcium.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline calcium is the crystalline calcium salt of a kind of oxytetracycline or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is equivalent to not less than 865 micrograms of oxytetracycline per milligram on an anhydrous basis.

(ii) It is nontoxic.

(iii) Its moisture content is not less than 8 percent and not more than 14 percent.

(iv) Its pH in an aqueous suspension containing 25 milligrams per milliliter is not less than 6.0 and not more than 8.0.

(v) Its calcium content as the sulfated ash is not less than 3.85 percent and not more than 4.35 percent on an anhydrous basis.

(vi) It gives a positive identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 (b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, toxicity, moisture, pH, calcium content, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 446.-65a(b) (1).

(2) *Toxicity.* Proceed as directed in § 436.33 of this chapter.

(3) *Moisture.* Proceed as directed in § 440.74a(b)(5) of this chapter.

(4) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using a saturated aqueous suspension containing 25 milligrams per milliliter.

(5) *Calcium content.* Place approximately 1 gram of the sample, accurately weighed, in a tared porcelain crucible, and carefully ignite at a low temperature until thoroughly charred. The crucible may be loosely covered with a porcelain lid during the charring. Add to the contents of the crucible 2.0 milliliters of sulfuric acid, and cautiously heat until white fumes are evolved, then ignite, preferably in a muffle furnace, at 500° C. to 600° C. until the carbon is all burned off. Cool the crucible in a desiccator and weigh. From the weight of residue obtained, calculate the calcium content as follows:

$$\text{Percent calcium} = \frac{\text{Weight of residue} \times 0.29435}{\text{Weight of sample}} \times 100.$$

(6) *Crystallinity.* Proceed as directed in § 440.80a(b)(5)(iii) of this chapter.

(7) *Identity.* Proceed as directed in § 446.65a(b)(10).

§ 446.67a Sterile oxytetracycline hydrochloride.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride is the crystalline hydrochloride salt of a kind of oxytetracycline or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 835 micrograms of oxytetracycline per milligram on an anhydrous basis.

(ii) It is sterile.

(iii) It is nontoxic.

(iv) It is nonpyrogenic.

(v) It contains no histamine nor histamine-like substance.

(vi) Its moisture content is not more than 2.0 percent.

(vii) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.0 and not more than 3.0.

(viii) When calculated on the anhydrous basis, its absorptivity at 353 millimicrons is 92.5±4.3 percent of that of the oxytetracycline standard similarly treated and corrected for potency.

(ix) It gives a positive result to an identity test for oxytetracycline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, toxicity, pyrogens, histamine, moisture, pH, absorptivity, crystallinity, and identity.

(ii) Samples of the batch:

(*a*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 446.65a(b)(1).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Toxicity.* Proceed as directed in § 440.80a(b)(4) of this chapter, using as a test dose 0.5 milliliter of a solution in sterile distilled water containing 2.0 milligrams of oxytetracycline per milliliter.

(4) *Pyrogens.* Proceed as directed in § 440.80a(b)(3) of this chapter, using as a test dose 1.0 milliliter per kilogram of a solution in sterile, pyrogen-free distilled water containing 5 milligrams of oxytetracycline per milliliter.

(5) *Histamine.* Proceed as directed in § 444.70a(b)(5) of this chapter, using as a test dose 0.6 milliliter per kilogram of a sodium chloride solution containing 5 milligrams of oxytetracycline per milliliter.

(6) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) of this chapter.

(7) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(8) *Absorptivity.* Proceed as directed in § 446.65a(b)(8).

(9) *Crystallinity.* Proceed as directed in § 440.80a(b)(5)(iii) of this chapter.

(10) *Identity.* Proceed as directed in § 446.65a(b)(10).

§ 446.75a Sterile rolitetracycline.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Sterile rolitetracycline is *N*-(1-pyrrolidinylmethyl) tetracycline. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram on the anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It passes the safety test.

(v) It contains no histamine nor histamine-like substances.

(vi) Its moisture content is not more than 3.0 percent.

(vii) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 7 and not more than 9, and such solution is substantially clear.

(viii) It is crystalline.

(ix) When calculated on an anhydrous basis, its absorptivity at 380 nanometers is 100±4.4 percent of that of the rolitetracycline standard similarly treated.

(x) It passes the identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, safety, histamine, moisture, pH, crystallinity, absorptivity, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient methyl alcohol to give a solution containing 1 milligram of rolitetracycline per milliliter (estimated). Further dilute an aliquot of this solution with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 0.25 microgram of rolitetracycline per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this subchapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this subchapter, using a solution containing 5.0 milligrams of rolitetracycline per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this subchapter.

(5) *Histamine.* Proceed as directed in § 436.35 of this subchapter.

(6) *Moisture.* Proceed as directed in § 436.201 of this subchapter.

(7) *pH.* Proceed as directed in § 436.202 of this subchapter, using an aqueous solution containing 10 milligrams per milliliter.

(8) *Crystallinity.* Proceed as directed in § 436.203(a) of this subchapter.

(9) *Absorptivity.* Determine the absorbance of the sample and standard solutions in the following manner: Dissolve an accurately weighed portion of approximately 40 milligrams each of the sample and standard in approximately 150 milliliters of distilled water and mix thoroughly. Dilute each to exactly 250 milliliters with distilled water and mix thoroughly. Transfer a 10.0-milliliter aliquot of each of these solutions to separate 100-milliliter volumetric flasks. Add approximately 75 milliliters of distilled water and 5.0 milliliters of 5*N* NaOH to each flask, and then dilute to volume with water and mix thoroughly. Exactly 6 minutes after the addition of the NaOH, determine the absorbance of each solution at 380 nanometers, using a suitable spectrophotometer and distilled water as the blank. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

$$\text{Percent relative absorptivity} = \frac{\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{weight of standard in milligrams}}{\text{weight of sample in milligrams} \times (100-m)} \times \frac{\text{potency of standard in micrograms per milligram}}{} \times 10}{}$$

where m = percent moisture in the sample.

(10) *Identity.* Place approximately 100 milligrams of the sample to be tested in a test tube, and 5 milliliters of 1*N* NaOH, and heat gently to boiling for about 15 seconds. (The musty, aminelike odor of pyrrolidine is detectable.) Allow to cool to room temperature. A deep burgundy-red color of the clear solution indicates the presence of rolitetracycline.

§ 446.76a Sterile rolitetracycline nitrate.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Sterile rolitetracycline nitrate is the nitrate salt of *N*-(pyrrolidinomethyl) tetracycline. It is so purified and dried that:

(i) It contains not less than 765 micrograms of rolitetracycline per milligram on an "as is" basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It passes the safety test.

(v) It contains no histamine nor histamine-like substances.

(vi) Its moisture content is not more than 5.0 percent.

(vii) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 3.5 and not more than 5.5.

(viii) It is crystalline.

(ix) When calculated on an anhydrous basis, its absorptivity at 380 nanometers is 89.2±4.0 percent of that of the rolitetracycline standard similarly treated and corrected for potency.

(x) It gives a positive result to the identity tests for rolitetracycline nitrate.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, safety, histamine, moisture, pH, crystallinity, absorptivity, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient methyl alcohol to give a solution containing 1 milligram of rolitetracycline per milliliter (estimated). Further dilute an aliquot of this solution with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 0.25 microgram of rolitetracycline per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this subchapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this subchapter, using a solution containing 5.0 milligrams of rolitetracycline per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this subchapter.

(5) *Histamine.* Proceed as directed in § 436.35 of this subchapter.

(6) *Moisture.* Proceed as directed in § 436.201 of this subchapter.

(7) *pH.* Proceed as directed in § 436.202 of this subchapter, using an aqueous solution containing 10 mfiligrams per milliliter.

(8) *Crystallinity.* Proceed as directed in § 436.203(a) of this subchapter.

(9) *Absorptivity.* Determine the absorbance of the sample and standard solutions in the following manner: Dissolve an accurately weighed portion of approximately 40 milligrams each of the sample and standard in approximately 150 milliliters of distilled water and mix thoroughly. Dilute each to exactly 250 milliliters with distilled water and mix thoroughly. Transfer a 10.0-milliliter aliquot of each of these solutions to representative 100-milliliter volumetric flasks. Add about 75 milliliters of distilled water and 5.0 milliliters of 5*N* NaOH to each and then dilute to volume with water and mix thoroughly. Exactly 6 minutes after the addition of the NaOH, determine the absorbance of each solution at 380 nanometers, using a suitable spectrophotometer and distilled water as the blank. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

$$\text{Percent relative absorptivity} = \frac{\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{weight of standard in milligrams}}{\text{weight of sample in milligrams} \times (100-m)} \times \text{potency of standard in micrograms per milligram} \times 10,}{}$$

where: m = percent moisture in the sample.

(10) *Identity—*(i) *Rolitetracycline.* Place approximately 100 milligrams of the sample to be used in a test tube, add 5 milliliters of 1*N* NaOH, and heat gently to boiling for about 15 seconds. (The musty, amine-like odor of pyrrolidine is detectable.) Allow to cool to room temperature. A deep burgundy-red color of the clear solution indicates the presence of rolitetracycline.

(ii) *Nitrate identity.* Transfer approximately 1 gram of sample to a 250-milliliter beaker, add 100 milliliters of water, and acidify with 1 milliliter of acetic acid. Heat to boiling and, with constant stirring, add 10 milliliters of a 10-percent solution of nitron (1,4-diphenyl-3,5-endo - anilino - 4,5 - dihydro - 1,2,4-triazole) $C_{20}H_{16}N_4$[7] in 1*N* acetic acid. Allow to cool. A heavy precipitate indicates the presence of nitrate.

§ 446.80 Tetracycline.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Tetracycline is the hydrated or anhydrous crystalline compound of the deschloro derivative of a kind of chlortetracycline, or a mixture of two or more such compounds. It is so purified that:

(i) Its potency is not less than 975 micrograms per milligram on the anhydrous basis.

(ii) It is nontoxic.

(iii) Its moisture content is not more than 13 percent.

(iv) Its pH in an aqueous suspension prepared by adding 10 milligrams per milliliter is not less than 3.0 and not more than 7.0.

(v) Its absorptivity at 380 mμ, calculated on the anhydrous basis, is 108.2±3.75 percent of the tetracycline hydrochloride working standard treated as described in § 446.81a(b)(5) and calculated on the anhydrous basis.

(2) *Packaging.* In all cases the immediate containers shall be tight containers as defined by the U. S. P. and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limits therefor in applicable standards, except that minor changes so caused that are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* Each package of tetracycline shall bear on its outside wrapper or container and the immediate container, as hereinafter indicated, the following:

(i) The batch mark.

(ii) The number of micrograms of tetracycline per milligram expressed in terms of its equivalency of tetracycline hydrochloride, and the total number of grams in the immediate container.

(iii) The statement "Expiration date ----------", the blank being filled in with the date which is 36 months after the month during which it was certified.

(iv) The statement "For use only in the manufacture of nonparenteral drugs".

(v) The statement "Caution: Federal law prohibits dispensing without prescription".

(4) *Request for certification, check tests and assays; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement

[7] Nitron is available from J. T. Baker Laboratory Chemicals, North Phillipsburg, N.J. 08865.

showing the batch mark, the number of packages of each size in the batch, and (unless it was previously submitted) the date on which the latest assay of the drug comprising the batch was completed. Such request shall be accompanied or followed by the results of tests and assays made by him on the batch for potency, toxicity, moisture, pH, crystallinity, and absorptivity.

(ii) Such person shall submit with his request an accurately representative sample of the batch consisting of 10 packages, each containing approximately 60 milligrams taken from a different part of such batch and each packaged in accordance with the requirements of paragraph (a)(2) of this section.

(iii) In connection with contemplated requests for certification of batches of another drug in the manufacture of which tetracycline is to be used, the manufacturer of the batch that is to be so used may request the Commissioner to make check tests and assays on a sample of such batch taken as prescribed by paragraph (a)(4)(ii) of this section. From the information required by paragraph (a)(4)(i) of this section may be omitted results of tests and assays not required for the batch when used in such other drug. The Commissioner shall report to such manufacturer the results of such check tests and assays as are so requested.

(b) *Tests and methods of assay*—(1) *Moisture.* Proceed as directed in § 440.74a(b)(5) of this chapter. Use the value obtained to calculate the weighed samples used in paragraph (b)(2), (3), and (6) of this section to the anhydrous compound.

(2) *Potency.* Using 40 milligrams (as the anhydrous compound) of sample, proceed as directed in § 446.81a(b)(1).

(3) *Toxicity.* Proceed as directed in § 444.70a(b)(3) of this chapter, using as a test dose 0.5 milliliter of an aqueous solution containing 2.0 milligrams per milliliter prepared by dissolving 40 milligrams (as the anhydrous compound) in 2.0 milliliters of 0.1*N* HCl and diluting with the required amount of water.

(4) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using an aqueous suspension prepared by adding 10 milligrams per milliliter.

(5) *Crystallinity.* Proceed as directed in § 440.80a(b)(5)(iii) of this chapter.

(6) *Absorptivity.* Proceed as directed in § 446.81a(b)(5), except dissolve 40 milligrams (as the anhydrous compound) in 2.0 milliliters of 0.1 *N* HCl and dilute to 250 milliliters with distilled water.

§ 446.81a Sterile tetracycline hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline hydrochloride is the crystalline hydrochloride salt of the deschloro derivative of a kind of chlortetracycline or a mixture of two or more such salts, with or without one or more suitable and harmless stabilizing agents. It is so purified and dried that:

(i) Its potency, and the potency of the tetracycline hydrochloride used in the manufacture of tetracycline hydrochloride intended for use by injection, is not less than 900 micrograms per milligram.

(ii) It is sterile.

(iii) It is nontoxic.

(iv) It is nonpyrogenic.

(v) It contains no histamine nor histamine-like substance.

(vi) Its moisture content is not more than 2 percent.

(vii) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 1.8 and not more than 2.8, except if it is intended for use by injection its pH is not less than 2.0 and not more than 3.0.

(viii) Its absorptivity and the absorptivity of the tetracycline hydrochloride used in the manufacture of tetracycline hydrochloride intended for use by injection is 100±4 percent of the tetracycline hydrochloride working standard similarly treated and both calculated on the anhydrous basis.

(2) *Packaging; labeling; request for certification, check tests and assays, samples.* Tetracycline hydrochloride conforms to all requirements and procedures prescribed for chlortetracycline hydrochloride by § 446.10a(a)(2), (3), and (4), except that:

(i) It shall be labeled with an expiration date that is 36 months after the month during which the batch was certified, except that it may be labeled with the date that is 48 months or 60 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time such drug as prepared by him complies with the standards prescribed by paragraph (a)(1) of this section.

(ii) The person who requests certification of a batch of tetracycline hydrochloride that contains stabilizing agents and is intended for use by injection shall submit with his request (unless they were previously submitted) the results of the latests tests and assays made on the batch of the tetracycline hydrochloride used in making such batch for potency and absorptivity and a sample consisting of two packages each containing approximately 500 milligrams of the tetracycline hydrochloride used in making such batch for potency and absorptivity.

(b) *Tests and methods of assay*—(1) *Potency.* Use the tetracycline hydrochloride working standard as the standard of comparison, and proceed as directed in § 446.10a(b)(1), except:

(i) Use 0.1*N* HCl instead of 0.01*N* HCl for the preparation of the standard stock solution and the sample stock solution.

(ii) In the cylinder-plate assay:

(*a*) Further dilute the samples in 0.1 *M* phosphate buffer, pH 4.5, to an estimated final concentration of 1.0 microgram per milliliter instead of 0.10 microgram per milliliter; and

(*b*) The final concentrations for the standard curve are 0.64, 0.8, 1.0, 1.25, and 1.56 micrograms per milliliter.

(iii) In the turbidimetric assay:

(*a*) Further dilute the samples in 0.1 *M* phosphate buffer, pH 4.5, to an estimated final concentration of 0.24 microgram per milliliter instead of 0.06 microgram per milliliter; and

(*b*) The final concentrations for the standard curve are 0.146, 0.187, 0.240, 0.308, and 0.395 microgram per milliliter.

The potency of tetracycline hydrochloride intended for use by injection is satisfactory if each immediate container contains not less than 90 percent of the tetracycline hydrochloride that it is represented to contain.

(2) *Sterility and toxicity.* Proceed as directed in § 446.10a(b)(2) and (3).

(3) *Pyrogens.* Proceed as directed in § 440.80a(b)(3) of this chapter, using as a test dose 1.0 milliliter per kilogram of a solution containing 5.0 milligrams of tetracycline hydrochloride per milliliter of sterile, pyrogen-free distilled water.

(4) *Histamine, moisture, pH, and crystallinity.* Proceed as directed in § 446.10a(b)(5), (6), and (7) and § 440.80a(b)(5)(iii) of this chapter.

(5) *Absorptivity.* Dissolve approximately 40 milligrams on the anhydrous basis of the sample, accurately weighed, in approximately 150 milliliters of distilled water by mixing thoroughly. Dilute to exactly 250 milliliters with distilled water and mix thoroughly. Transfer a 10.0-milliliter aliquot of this solution to a 100-milliliter volumetric flask, add about 75 milliliters of distilled water and 5.0 milliliters of 5 *N* NaOH, and then make to 100 milliliters with water and mix thoroughly. Exactly 6 minutes after the addition of the NaOH, determine the absorbance of the solution at 380 mμ compared with distilled water as a blank. Use a suitable spectrophotometer for the absorbance measurements.

$$\text{Absorptivity (1\%, 1 cm.)} = \frac{\text{Absorbance at 380 m}\mu \times 25{,}000}{\text{Weight of sample in milligrams}}$$

§ 446.82 Tetracycline phosphate complex.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline phosphate complex is the crystalline sodium metaphosphate complex of a kind of tetracycline. It is so purified and dried that:

(i) Its potency is not less than 750 μg. per milligram on the anhydrous basis.

(ii) It is nontoxic.

(iii) Its moisture content is not more than 9 percent.

(iv) Its pH in an aqueous suspension prepared by adding 10 milligrams per milliliter is not less than 2.0 and not more than 4.0.

(v) Its absorptivity at 380 mμ, calculated on the anhydrous basis, is 82.0±4.9 percent of the tetracycline hydrochloride working standard treated as described in § 446.81a(b)(5).

(vi) It passes identity tests showing a presence of phosphate, a content of not more than 0.2 percent chloride, and a content of not more than 1 percent tetracycline base.

(2) *Packaging; labeling; request for certification, samples.* Tetracycline phosphate complex conforms to all requirements and procedures prescribed for tetracycline by § 446.80(a) (2), (3), and (4), except that it shall be labeled with an expiration date that is 18 months after the month during which the batch was certified, and except that the sample submitted for certification shall contain one additional package containing approximately 1 gram.

(b) *Tests and methods of assay*—(1) *Moisture, potency, toxicity, pH, crystallinity, absorptivity.* Proceed as directed in § 446.80(b) (1), (2), (3), (4), (5), and (6).

(2) *Identity.* Proceed as directed in paragraph (b)(2) (i), (ii), and (iii) of this section.

(i) *Presence of phosphate.* Suspend 100 milligrams of the sample in 10 milliliters of distilled water and filter a small portion by gravity. Transfer 1 milliliter to a 100-milliliter glass-stoppered cylinder, add 10 milliliters of distilled water, 2 milliliters of ammonium molybdate test solution, 1 milliliter of stannous chloride test solution, and 10 milliliters of isobutyl alcohol-benzene mixture (1:1 ratio), all in the order named. Shake vigorously for 1 minute, allow the layers to separate, and examine the top organic layer. In the presence of phosphate, the top layer turns blue.

(ii) *Chloride content.* To 1 milliliter of the filtrate prepared as directed in the first sentence of paragraph (b)(2)(i) of this section, add 1 drop of silver nitrate test solution and 1 drop of nitric acid. Any turbidity produced is not greater than that obtained by similarly treating 1 milliliter of 0.057*N* hydrochloric acid.

(iii) *Determination of percent tetracycline base.* This test is used to determine the quantity of tetracycline present as base in mixtures with phosphate salts.

(*a*) *Reagents.* (*1*) 1,4-Dioxane.

(2) Purified dioxane: Pass the dioxane through a column of Amberlite IRA 400 (OH^-) resin.

(3) Perchloric acid, 0.01*N*: Dilute 0.84 milliliter of 70 percent perchloric acid to 1,000 milliliters with purified dioxane; standardize at least once every 2 days, as follows: Weigh accurately about 70 milligrams of diphenylguanidine, and dissolve in 50 milliliters of ethyl alcohol in a 250-milliliter flask. Add two drops of methyl red, and titrate with the perchloric acid solution from a burette until the yellow color changes to orange. Deduct the volume of the perchloric acid consumed by 50 milliliters of the ethyl alcohol, and calculate the normality. Each 2.113 milligrams of diphenylguanidine is equivalent to 1 milliliter of 0.01*N* perchloric acid.

(*4*) Methyl red indicator: Dissolve 100 milligrams of methyl red in 100 milliliters of methyl alcohol.

(*b*) *Procedure.* Place an accurately weighed 1-gram sample in a 50-milliliter Erlenmeyer flask, add 10.0 milliliters of purified dioxane and shake the mixture manually for about 2 minutes. Allow to settle, decant all the supernatant liquid into a 50-milliliter polyethylene centrifuge tube, cover with Parafilm, and centrifuge until clear (about 3 minutes). Pipette 5.0 milliliters of the clear, supernatant solution into a 50-milliliter beaker, stir magnetically, and titrate with 0.01*N* perchloric acid, using methyl red as the indicator. The endpoint is the last color change to orange when a drop of titrant is added.

Calculation:

$$\text{Percent tetracycline base} = \frac{\text{Milliliters of acid used} \times \text{normality} \times 0.4445 \times 200}{\text{Weight of sample}}$$

The sample is satisfactory if the results from the tests described indicate that there is a presence of phosphate, an absence of chloride, and not more than 1 percent of tetracycline base present.

Subpart B—Oral Dosage Forms

§ 446.110 Chlortetracycline hydrochloride oral dosage forms.

§ 446.110a Chlortetracycline hydrochloride tablets; tetracycline hydrochloride tablets; tetracycline tablets.

(a) *Requirements for certification.* (1) Chlortetracycline hydrochloride tablets, tetracycline hydrochloride tablets, and tetracycline tablets are tablets that conform to all requirements and are subject to all procedures prescribed by § 446.110b(a) for chlortetracycline hydrochloride capsules, tetracycline hydrochloride capsules, and tetracycline capsules, except that:

(i) The average moisture content of the tablets is not more than 3 percent, unless the person who requests certification has submitted to the Commissioner information adequate to prove that his drug is stable when it has a moisture content not exceeding 6 percent.

(ii) In addition to the requirements prescribed by § 446.110b(a), tablets not exceeding 15 milliliters in diameter, or not intended only for use in preparing solutions, shall disintegrate within 1 hour. A person who requests certification shall therefore also submit for disintegration-time studies, results of this test made by him and a sample of six tablets.

(2) [Reserved]

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 446.110b(b)(1). The average potency of the drug is satisfactory if it contains not less than 85 percent of the number of milligrams it is represented to contain.

(2) *Moisture.* Proceed as directed in § 436.200(b) or § 436.201 of this chapter.

(3) *Disintegration time.* Proceed as directed in § 440.180a(b)(3) of this chapter.

§ 446.110b Chlortetracycline hydrochloride capsules; tetracycline hydrochloride capsules; tetracycline capsules; tetracycline phosphate complex capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chlortetracycline hydrochloride capsules, tetracycline hydrochloride capsules, tetracycline capsules and tetracycline phosphate complex capsules are capsules composed of crystalline chlortetracycline hydrochloride, tetracycline hydrochloride, tetracycline, or tetracycline phosphate complex, with or without one or more suitable and harmless buffer substances, vegetable oils, preservatives, diluents, binders, lubricants, colorings, and flavorings and glucosamine hydrochloride enclosed in a gelatin capsule. Each capsule shall contain not less than 50 milligrams of chlortetracycline hydrochloride, tetracycline hydrochloride, tetracycline, or tetracycline phosphate complex. Its moisture content is not more than 2 percent if it contains chlortetracycline hydrochloride, not more than 3 percent if it contains tetracycline (not more than 9 percent if it contains sodium or potassium metaphosphate as a buffer substance), not more than 4 percent if it contains tetracycline hydrochloride, and not more than 9 percent if it contains tetracycline phosphate complex. The chlortetracycline hydrochloride used conforms to the requirements of § 446.10a(a)(1), except § 446.10a(a)(1)(ii), (iv), and (v). The tetracycline hydrochloride used conforms to the requirements of § 446.81a(a)(1), except § 446.81a(a)(1)(ii), (iv), and (v). The tetracycline used conforms to the requirements of § 446.80(a)(1). The tetracycline phosphate complex used conforms to the requirements of § 446.82(a). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* Unless each capsule is enclosed in a foil or plastic film and such enclosure is a tight container as defined by the U.S.P., except the provision that it shall be capable of tight reclosure, the immediate container shall be a tight container as so defined. The immediate container may also contain a desiccant separated from the capsules by a plug of cotton or other like material. The composition of the immediate container, or of the foil or film enclosure, shall be such as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on the outside wrapper or container and the immediate container, as hereinafter indicated, the following:

(i) The statement "Expiration date _____", the blank being filled in with one of the following dates after the month during which the batch was certified:

(*a*) If chlortetracycline hydrochloride is used, 60 months.

(*b*) If tetracycline hydrochloride is used, 48 months, except that the date that is 60 months may be used if the person who requests certification has submitted to the Commissioner results of tests and assay showing that after having been stored for such period of time such drug as prepared by him complies with the standards prescribed therefor by paragraph (a) (1) of this section.

(*c*) If tetracycline is used, 36 months.

(*d*) If tetracycline phosphate complex is used, 24 months, except that the blank may be filled in with the date that is 36 months, 48 months, or 60 months, after the month in which the batch was certified, if the person who requests certification has submitted to the Commisisoner results of tests and assays showing that after having been stored for such period of time such drug as prepared by him complies with the standards prescribed by paragraph (a) (1) of this section.

(*e*) If it contains sodium metaphosphate, 36 months.

(ii) [Reserved]

(4) *Requests for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless it was previously submitted) the date on which the latest assay of the chlortetracycline, tetracycline hydrochloride, tetracycline or tetracycline phosphate complex used in making such batch was completed, the number of milligrams in each capsule, the quantity of each ingredient used in making the batch, the date on which the latest assay of the drug comprising such batch was completed, and a statement that each ingredient used in making the batch conforms to the requirements prescribed therefore, if any, by this section.

(ii) Except as otherwise provided in paragraph (a) (4) (iv) of this section, such person shall submit in connection with his request results of the tests and assays listed after each of the following made by him on an accurately representative sample of:

(*a*) The batch; average potency per capsule and average moisture.

(*b*) The chlortetracycline, tetracycline hydrochloride, tetracycline, or tetracycline phosphate complex used in making the batch: Potency, toxicity, moisture, pH, crystallinity, absorptivity (if it is tetracycline hydrochloride, tetracycline, or tetracycline phosphate complex) and identity if it is tetracycline phosphate complex.

(iii) Except as otherwise provided by paragraph (a) (4) (iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch; one capsule for each 5,000 capsules in the batch, but in no case less than 30 capsules, collected by taking single capsules at such intervals throughout the entire time of preparation that the quantities encapsulated during the intervals are approximately equal.

(*b*) The chlortetracycline, tetracycline hydrochloride, tetracycline, or tetracycline phosphate complex used in making the batch: 10 packages, each containing approximately equal portions of not less than 60 milligrams, and if it is tetracycline phosphate complex, one additional package containing approximately 1 gram. Each such package shall be packaged in accordance with the requirements of § 446.10a(a) (2).

(*c*) In case of an initial request for certification, each other ingredient used in making the batch; one package of each containing approximately 5 grams.

(iv) No result referred to in paragraph (a) (4) (ii) (*b*) of this section, and no sample referred to in paragraph (a) (4) (iii) (*b*) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay*— (1) *Potency.* Using 3 capsules of 250 milligrams or 5 capsules of 50 milligrams or 100 milligrams and 500 milliliters of 0.01*N* HCl in the blender, proceed as directed in § 436.513(a) if it is chlortetracycline. If it is tetracycline hydrochloride, tetracycline, or tetracycline phosphate complex, use 500 milliliters of 0.1 *N* HCl, and proceed as directed in § 446.81a(b) (1). If it contains vegetable oils, use 1 milliliter of polysorbate 80 and sufficient solvent to give 500 milliliters. The average potency of the drug is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 440.80a(b) (5) (i) or § 440.74a(b) (5) of this chapter.

§ 446.111 Chlortetracycline calcium sirup (chlortetracycline calcium oral drops); tetracycline sirup (tetracycline oral drops); tetracycline magnesium sirup (tetracycline magnesium oral drops).

(a) *Requirements for certification*— (1) *Standards of identity, strength, quality, and purity.* Chlortetracycline calcium sirup, tetracycline sirup, and tetracycline magnesium sirup are sirups that contain chlortetracycline calcium prepared from crystalline chlortetracycline hydrochloride, tetracycline, or tetracycline magnesium prepared from tetracycline or tetracycline hydrochloride with or without one or more suitable glucosamine hydrochloride, *N*-acetylglucosamine, and one or more suitable and harmless buffer substances, suspending and stabilizing agents, and preservatives, suspended in a suitable and harmless vehicle. Each milliliter shall contain a quantity of chlortetracycline calcium or tetracycline or tetracycline magnesium equivalent to not less than 25 milligrams or chlortetracycline hydrochloride or tetracycline hydrochloride. The pH is not less than 6.5 nor more than 9.0, except if it is tetracycline sirup that pH is not less than 3.5 nor more than 6.0, except if it contains *N*-acetylglucosamine the pH is not less than 5.0 and not more than 7.5. The crystalline chlortetracycline hydrochloride used conforms to the requirements of § 446.10a(a) (1), except § 446.10a(a) (1) (ii), (iv), and (v). The crystalline tetracycline used conforms to the requirements of § 446.80(a). The tetracycline hydrochloride used conforms to the requirements of § 446.81a(a) (1) except § 446.81a(a) (1) (ii), (iv) and (v). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* In all cases the immediate container shall be a tight container as defined by the U.S.P. and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the outside wrapper or container and the immediate container, the statement "Expiration date _____", the blank being filled in with the date that is 12 months after the month during which the batch was certified, except the blank may be filled in with the date that is 18 months, 24 months, 36 months, or 48 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time such drug as prepared by him complies with the standards prescribed by paragraph (a) (1) of this section.

(ii) If it contains, in addition to chlortetracycline calcium, tetracycline, or tetracycline magnesium, one or more of the other active ingredients specified in paragraph (a) (1) of this section, after the name "chlortetracycline calcium sirup" or "tetracycline sirup" or "tetracycline magnesium sirup," wherever it appears, the words "with _____ (the blank being filled in with the established name of such other ingredient)," in juxtaposition with such name.

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless it was previously submitted) the date on which the latest assay of the chlortetracycline hydrochloride or tetracycline or tetracycline hydrochloride used in making such batch was completed, the date on which the latest assay of the drug comprising such batch was completed, the quantity of each ingredient used in making the batch, and a statement that each such ingredient conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request results of the tests and assays listed after each of the following, made by him on an accurately representative sample of:

(*a*) The batch: Average potency per milliliter, pH, and toxicity if it is chlortetracycline calcium sirup or tetracycline magnesium sirup.

(*b*) The chlortetracycline hydrochloride or tetracycline or tetracycline hydrochloride used in making the batch; potency, toxicity, moisture, pH, crystallinity, and absorptivity if it is tetracycline or tetracycline hydrochloride.

(iii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch; one package for each 5,000 packages in the batch, but in no case less than 5 packages, collected by taking single packages at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(*b*) The chlortetracycline hydrochloride or tetracycline or tetracycline hydrochloride used in making the batch; 10 packages each containing approximately equal portions of not less than 60 milligrams, packaged in accordance with the requirements of § 446.10a(a)(2).

(*c*) In case of an initial request for certification, each other ingredient used in making the batch; one package of each containing approximately 5 grams.

(iv) No result referred to in paragraph (a)(4)(ii)(*b*) of this section, and no sample referred to in paragraph (a)(4)(iii)(*b*) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay*—(1) *Potency.* If it is chlortetracycline calcium sirup, proceed as directed in § 446.10a(b)(1). If it is tetracycline sirup or tetracycline magnesium sirup, use a 1-milliliter aliquot of the sample and proceed as directed in § 446.510b(b)(1). Blend for 5 minutes and proceed as directed in § 446.81a(b)(1). Its potency is satisfactory if it contains not less than 85 percent of the equivalent number of milligrams of chlortetracycline or tetracycline hydrochloride per milliliter that it is represented to contain.

(2) *pH.* Using the undiluted sample, proceed as directed in § 440.80a(b)(5)(ii) of this chapter.

(3) *Toxicity (if it is chlortetracycline calcium sirup or tetracycline magnesium sirup).* Administer orally, by means of cannula or other suitable device, to each of 5 mice, within the weight range of 18 grams to 25 grams, 0.5 milliliter containing 12.5 milligrams, except that if it contains analgesic substances or antihistaminics, administer 0.5 milliliter containing 6.25 milligrams. Dilute the preparation with sterile distilled water, if necessary, to contain the quantity of drug per 0.5 milliliter. If no animal dies within 48 hours, the sample is nontoxic. If one or more animals die within 48 hours, repeat the test, using for each test five or more previously unused mice weighing 20 grams (±0.5 grams) each; if the total deaths within 48 hours is no greater than 10 percent of the total number of animals tested, including the original test, the sample is nontoxic.

§ 446.115 Demeclocycline oral dosage forms.

§ 446.115a Demeclocycline oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Demeclocycline oral suspension is composed of demeclocycline with or without one or more suitable and harmless buffer substances, suspending and stabilizing agents, and preservatives suspended in a suitable and harmless vehicle. Each milliliter contains demeclocycline equivalent to either 15 milligrams or 60 milligrams of demeclocycline hydrochloride. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of demeclocycline hydrochloride equivalent that it is represented to contain. The pH is not less than 4 and not more than 5.8. The demeclocycline used conforms to the standards prescribed by § 446.15(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The demeclocycline used in making the batch for potency, safety, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The demeclocycline used in making the batch: 10 packages, each containing approximately 250 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Transfer an accurately measured representative portion of the sample to an appropriate-sized volumetric flask, dilute to volume with 0.1*N* hydrochloric acid, and mix. Further dilute an aliquot with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 0.100 microgram of demeclocycline hydrochloride per milliliter (estimated).

(2) pH. Proceed as directed in § 436.202 of this chapter, using the undiluted sample.

§ 446.115b Demeclocycline for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Demeclocycline for oral suspension is composed of demeclocycline with or without one or more suitable and harmless buffer substances, preservatives, diluents, colorings, and flavorings. When reconstituted as directed in the labeling, each milliliter contains demeclocycline equivalent to 15 milligrams of demeclocycline hydrochloride. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of demeclocycline hydrochloride equivalent that it is represented to contain. Its moisture content is not more than 5 percent. The demeclocycline used conforms to the standards prescribed by § 446.15(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The demeclocycline used in making the batch for potency, safety, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The demeclocycline used in making the batch: 10 packages, each containing approximately 250 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Transfer an accurately measured representative portion of the sample to an appropriate-sized volumetric flask, dilute to volume with 0.1*N* hydrochloric acid, and mix. Further dilute an aliquot with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 0.100 microgram of demeclocycline hydrochloride per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 446.115c Demeclocycline-nystatin for oral suspension.

(a) *Requirements for certification.* Demeclocycline-nystatin for oral suspension conforms to all requirements and procedures prescribed by § 446.115b(a) for demeclocycline for oral suspension, except that:

(1) When reconstituted as directed in the labeling, each milliliter of oral suspension shall contain a quantity of demeclocycline equivalent to not less than 15 milligrams of demeclocycline hydrochloride and not less than 25,000 units of nystatin. The nystatin used conforms to the standards prescribed therefor by § 446.181b(a)(1).

(2) The expiration date of the drug shall be 12 months.

(3) In addition to complying with the requirements of § 446.115b(a)(3), a person who requests certification of a batch shall submit with his request a statement showing the batch mark and (unless they were previously submitted) the results and the date of the latest tests and assays of the nystatin used in making the batch for potency, toxicity, pH, moisture, and identity. He shall submit in connection with his request a sample consisting of one package

for each 5,000 packages in the batch, but in no case less than 6 packages, collected by taking single packages at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal, and (unless it was previously submitted) a sample consisting of 10 packages, each containing approximately equal portions of not less than 300 milligrams of the nystatin used in making the batch.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Demeclocycline content.* Proceed as directed in § 446.115b(b)(1).

(ii) *Nystatin content.* Reconstitute as directed in the labeling. Transfer an appropriate aliquot (usually from 1.0 milliliter to 5.0 milliliters) to a blending jar containing 150 milliliters of dimethylformamide. Blend for 2 minutes in a high-speed blender and then dilute an aliquot with sufficient dimethylformamide to give a concentration of 400 units per milliliter (estimated). Further dilute this solution with 10 percent phosphate buffer, pH 6.0, to give a concentration of 20 units per milliliter (estimated). Proceed as directed in § 446.181b(b)(2). Its content of nystatin is satisfactory if it contains not less than 85 percent of the number of units that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) or § 440.74a(b)(5) of this chapter.

Note: § 446.115c (a) and (b) (formerly §§ 146c.263 and 141c.263 respectively) were revoked at 34 FR 18161, Nov. 13, 1969. The effective date of the revocation was stayed at 34 FR 20427, Dec. 31, 1969; and further postponed at 35 FR 5811, Apr. 9, 1970.

§ 446.116 Demeclocycline hydrochloride oral dosage forms.

§ 446.116a Demeclocycline hydrochloride tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Demeclocycline hydrochloride tablets are composed of demeclocycline hydrochloride with one or more suitable and harmless diluents, lubricants, binders, and flavorings. Each tablet contains 75 milligrams, 150 milligrams, or 300 milligrams of demeclocycline hydrochloride. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of demeclocycline hydrochloride that it is represented to contain. Its loss on drying is not more than 2 percent. It shall disintegrate within 30 minutes. The demeclocycline hydrochloride used conforms to the standards prescribed by § 446.16(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The demeclocycline hydrochloride used in making the batch for potency, safety, loss on drying, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency, loss on drying, and disintegration time.

(ii) Samples required:

(*a*) The demeclocycline hydrochloride used in making the batch: 10 packages, each containing approximately 250 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of tablets into a high-speed glass blender jar containing sufficient 0.1*N* hydrochloric acid to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 0.100 microgram of demeclocycline hydrochloride per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter.

§ 446.116b Demeclocycline hydrochloride-nystatin tablets.

(a) *Requirements for certification.* Demeclocycline hydrochloride-nystatin tablets are tablets that conform to all the requirements and are subject to all procedures prescribed by § 446.116d(a) for demeclocycline hydrochloride-nystatin capsules, except that:

(1) Each tablet contains 300 milligrams of demeclocycline hydrochloride and 500,000 units of nystatin.

(2) The moisture content is not more than 4 percent.

(3) It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(4) In addition to the requirements prescribed by § 446.116d(a), demeclocycline hydrochloride-nystatin tablets shall disintegrate within 1 hour. A person who requests certification shall therefor also submit, for disintegration time studies, results of this test by him and a sample of six tablets.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Demeclocycline hydrochloride content.* Proceed as directed in § 446.116a(b)(1).

(ii) *Nystatin content.* Proceed as directed in § 449.150a(b)(1) of this chapter. Its content of nystatin is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units thereof that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 440.180a(b)(3) of this chapter.

Note: § 446.116b (a) and (b) (formerly § 146c.271 and § 141c.271 respectively) were revoked at 34 FR 18161, Nov. 13, 1969. The effective date of the revocation was stayed at 34 FR 20427, Dec. 31, 1969; and further postponed at 35 FR 5811, Apr. 9, 1970.

§ 446.116c Demeclocycline hydrochloride capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Demeclocycline hydrochloride capsules are composed of demeclocycline hydrochloride, with one or more suitable and harmless diluents and lubricants, enclosed in a gelatin capsule. Each capsule contains 75 milligrams, 150 milligrams, or 300 milligrams of demeclocycline hydrochloride. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of demeclocycline hydrochloride that it is represented to contain. Its loss on drying is not more than 2 percent, except that if starch is used as a diluent the loss on drying is not more than 8 percent. The demeclocycline hydrochloride used conforms to the standards prescribed by § 446.16(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The demeclocycline hydrochloride used in making the batch for potency, safety, loss on drying, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency and loss on drying.

(ii) Samples required:

(*a*) The demeclocycline hydrochloride used in making the batch: 10 packages, each containing approximately 250 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar containing sufficient 0.1*N* hydrochloric acid to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 0.100 microgram of demeclocycline hydrochloride per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

§ 446.116d Demeclocycline hydrochloride-nystatin capsules.

(a) *Requirements for certification.* Demeclocycline hydrochloride-nystatin capsules are capsules that conform to all requirements and are subject to all procedures prescribed by § 446.116c(a) for demeclocycline hydrochloride capsules, except that:

(1) Each capsule contains not less than 250,000 units of nystatin. The nystatin used conforms to the requirements prescribed therefor by § 446.181b(a)(1).

(2) The average moisture content of the capsules is not more than 5.0 percent.

(3) Its expiration date shall be the date that is 18 months after the month during which the batch was certified.

(4) In addition to complying with the requirements of § 446.116c(a)(3), a person who requests certification of a batch shall submit with his request a statement

showing the batch mark and (unless they were previously submitted) the results and the date of the latest tests and assays of the nystatin used in making the batch for potency, toxicity, pH, moisture, and identity. He shall also submit in connection with his request (unless it was previously submitted) a sample consisting of 10 packages, each containing approximately equal portions of not less than 300 milligrams of the nystatin used in making the batch.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Demeclocycline hydrochloride content.* Proceed as directed in § 446.116c(b)(1).

(ii) *Nystatin content.* Proceed as directed in § 446.181b(b)(1)(i)(*b*). Its content of nystatin is satisfactory if it contains not less than 85 percent of the number of units that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) of this chapter.

NOTE: § 446.116d (a) and (b) (formerly § 146c.259 and § 141c.259 respectively) were revoked at 34 FR 18161, Nov. 13, 1969. The effective date of the revocation was stayed at 34 FR 20427, Dec. 31, 1969; and further postponed at 35 FR 5811, Apr. 9, 1970.

§ 446.120 Doxycycline hyclate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Doxycycline hyclate capsules are composed of doxycycline hyclate and one or more suitable and harmless lubricants and diluents enclosed in a gelatin capsule. Each capsule contains doxycycline hyclate equivalent to either 50 or 100 milligrams of doxycycline. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of doxycycline that it is represented to contain. The moisture content is not more than 5.0 percent. It passes the identity test for the presence of the doxycycline moiety. The doxycycline hyclate used conforms to the standards prescribed by § 446.20.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The doxycycline hyclate used in making the batch for potency, safety, moisture, pH, doxycycline content, identity, and crystallinity.

(*b*) The batch for potency, moisture, and identity.

(ii) Samples required:

(*a*) The doxycycline hyclate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 36 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Blend a representative number of capsules in a high-speed glass blender with 0.1*N* hydrochloric acid and further dilute with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 0.100 microgram of doxycycline per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Identity.* Proceed as directed in § 436.308 of this chapter, except prepare the standard and sample solutions as follows: Dissolve precise amounts of the doxycycline capsule contents and of the doxycycline working standard in methanol and further dilute each solution to a concentration of 1 milligram of doxycycline per milliliter. Prepare the sample-standard mixed solution by mixing equal volumes of the final standard and sample solutions. The standard and sample must each produce a major, yellow fluorescent spot with the same *Rf* value, and the standard-sample mixed solution must show no separation of major spots.

§ 446.121 Doxycycline monohydrate for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Doxycycline monohydrate for oral suspension is doxycycline monohydrate with one or more suitable and harmless buffer substances, preservatives, diluents, colorings, and flavorings. Its moisture content is not more than 3 percent. It passes the identity test for the presence of the doxycycline moiety. When prepared as directed in the labeling, each milliliter contains the equivalent of 5 milligrams of doxycycline and its pH is not less than 5.0 and not more than 6.5. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of doxycycline that it is represented to contain. The doxycycline monohydrate used conforms to the standards prescribed by § 446.21.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The doxycycline monohydrate used in making the batch for potency, safety, moisture, pH, doxycycline content, identity, and crystallinity.

(*b*) The batch for potency, moisture, pH, and identity.

(ii) Samples required:

(*a*) The doxycycline monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Using a suitable syringe, transfer an appropriate aliquot of the suspension to a volumetric flask and dissolve with 0.1*N* hydrochloric acid. Further dilute with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 0.100 microgram of doxycycline per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Reconstitute as directed in the labeling and proceed as directed in § 436.202 of this chapter, using the undiluted sample.

(4) *Identity.* Proceed as directed in § 436.308 of this chapter, except prepare the standard and sample solutions as follows: Dissolve precise amounts of the doxycycline monohydrate for oral suspension and of the doxycycline working standard in methanol and further dilute each solution to a concentration of 1 milligram of doxycycline per milliliter. Prepare the sample-standard mixed solution by mixing equal volumes of the final concentration of the sample and standard solutions. The sample and standard must each produce a major, yellow fluorescent spot with the same R_f value and the sample-standard mixed solution must show no separation of major spots.

§ 446.150 Methacycline hydrochloride oral dosage forms.

§ 446.150a Methacycline hydrochloride capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Methacycline hydrochloride capsules are composed of methacycline hydrochloride and one or more suitable and harmless lubricants and diluents enclosed in a gelatin capsule. Each capsule contains methacycline hydrochloride equivalent to either 70 milligrams of methacycline, 140 milligrams of methacycline, or 280 milligrams of methacycline. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of methacycline that it is represented to contain. The moisture content is not more than 5.0 percent. The methacycline hydrochloride used conforms to the standards prescribed by § 446.50(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The methacycline hydrochloride used in making the batch for potency, safety, moisture, pH, absorptivity, identity, and crystallinity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The methacycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Blend a representative number of capsules in a high-speed glass blender with 0.01*N* methanolic hydrochloric acid (solution 13) and further dilute with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference

concentration of 0.06 microgram of methacycline per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 446.150b Methacycline hydrochloride sirup.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Methacycline hydrochloride sirup contains methacycline hydrochloride and one or more suitable and harmless buffers, dispersants, diluents, colorings, flavorings, and preservatives. It contains methacycline hydrochloride equivalent to 14 milligrams of methacycline per milliliter. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of methacycline that it is represented to contain. Its pH is not less than 6.5 nor more than 8.0. The methacycline hydrochloride used conforms to the standards prescribed by § 446.50(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The methacycline hydrochloride used in making the batch for potency, safety, moisture, pH, absorptivity, identity, and crystallinity.

(*b*) The batch for potency and pH.

(ii) Samples required.

(*a*) The methacycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Remove an appropriate aliquot of the sirup with a suitable syringe and dissolve with 0.01*N* methanolic hydrochloric acid (solution 13). Further dilute with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 0.06 microgram of methacycline per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter using the undiluted sample.

§ 446.160 Minocycline hydrochloride oral dosage forms.

§ 446.160a Minocycline hydrochloride tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Minocycline hydrochloride tablets are composed of minocycline hydrochloride and one or more suitable and harmless diluents, binders, lubricants, coloring, and coating substances. Each tablet contains minocycline hydrochloride equivalent to 100 milligrams of minocycline. Its potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of minocycline that it is represented to contain. Its moisture content is not more than 12 percent. The tablets disintegrate within 30 minutes. The minocycline hydrochloride used conforms to the standards prescribed by § 446.60(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The minocycline hydrochloride used in making the batch for potency, safety, moisture, pH, minocycline content, identity, and crystallinity.

(*b*) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(*a*) The minocycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of tablets into a high-speed glass blender jar containing sufficient 0.1*N* hydrochloric acid to give a stock solution of convenient concentration containing not less than 150 micrograms of minocycline per milliliter (estimated). Blend for 3 to 5 minutes. Remove an aliquot and further dilute with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 0.100 microgram of minocycline per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter, using the procedure described in paragraph (e)(1) of that section.

§ 446.160b Minocycline hydrochloride capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Minocycline hydrochloride capsules are composed of minocycline hydrochloride and one or more suitable and harmless lubricants and diluents enclosed in a gelatin capsule. Each capsule contains minocycline hydrochloride equivalent to 50 or 100 milligrams of minocycline. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of minocycline that it is represented to contain. Its moisture content is not more than 12 percent. The minocycline hydrochloride used conforms to the standards prescribed by § 446.60(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The minocycline hydrochloride used in making the batch for potency, safety, moisture, pH, minocycline content, identity, and crystallinity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The minocycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar with sufficient 0.1*N* hydrochloric acid to give a stock solution of convenient concentration containing not less than 150 micrograms of minocycline per milliliter (estimated). Blend for 3 to 5 minutes. Remove an aliquot and further dilute with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 0.100 microgram of minocycline per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 446.160c Minocycline hydrochloride oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Minocycline hydrochloride oral suspension is minocycline hydrochloride with one or more suitable flavorings, wetting agents, preservatives, and diluents in an aqueous vehicle. Each milliliter contains minocycline hydrochloride equivalent to 10 milligrams of minocycline. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of minocycline that it is represented to contain. Its pH is not less than 7.0 and not more than 9.0. The minocycline hydrochloride used conforms to the standards prescribed by § 446.60(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The minocycline hydrochloride used in making the batch for potency, safety, moisture, pH, minocycline content, identity, and crystallinity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The minocycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately measured representative aliquot of the well shaken suspension in sufficient 0.1*N* hydrochloric acid to give a stock solution of convenient concentration containing

not less than 150 micrograms of minocycline per milliliter (estimated). Remove an aliquot and dilute with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 0.100 microgram of minocycline per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this subchapter, using the undiluted sample.

§ 446.165 Oxytetracycline oral dosage forms.

§ 446.165a Oxytetracycline tablets.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline tablets are tablets composed of oxytetracycline, and one or more suitable and harmless, diluents, binders, lubricants, colorings, and coating substances. The potency of each tablet is 250 milligrams of oxytetracycline. The moisture content is not more than 7.5 percent. They shall disintegrate within 1 hour. The oxytetracycline used conforms to the standards prescribed by § 446.65a(a)(1)(i), (iii), (vi), (vii), (viii), and (ix). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline used in making the batch for potency, toxicity, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(*a*) The oxytetracycline used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except disintegration time: A minimum of 30 tablets.

(*2*) For distintegration time: Six tablets.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 446.65a (b)(1) (i) or (ii), except prepare the sample as follows: Place a representative number of tablets in a glass blending jar containing 500 milliliters of 0.1*N* hydrochloric acid. Using a high-speed blender, blend for at least 5 minutes and then make the proper estimated dilutions in 0.1*M* potassium phosphate buffer, pH 4.5, to the prescribed reference concentration. Its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of oxytetracycline that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 440.74a(b)(5) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 440.180a(b)(3) of this chapter.

§ 446.165b Oxytetracycline capsules; oxytetracycline hydrochloride capsules.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline capsules and oxytetracycline hydrochloride capsules are gelatin capsules containing oxytetracycline or oxytetracycline hydrochloride, with or without one or more suitable and harmless vegetable oils, buffers, preservatives, diluents, binders, and lubricants. They may contain glucosamine hydrochloride. The potency of each capsule is 50 milligrams, 100 milligrams, 125 milligrams or 250 milligrams of oxytetracycline. The moisture content is not more than 5.0 percent if it contains oxytetracycline hydrochloride and not more than 7.5 percent if it contains oxytetracycline. The oxytetracycline used conforms to the standards prescribed by § 446.65a(a)(1) (i), (iii), (vi), (vii), (viii), and (ix). The oxytetracycline hydrochloride used conforms to the standards prescribed by § 446.67a (a)(1) (i), (iii), (vi), (vii), (viii), and (ix). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline or oxytetracycline hydrochloride used in making the batch for potency, toxicity, moisture, pH, absorptivity, identity, and crystallinity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) Oxytetracycline or oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: Minimum 30 capsules.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency.* Using a representative number of capsules (usually 5 to 12) and 500 milliliters of 0.1*N* hydrochloric acid in a blender, proceed as directed in § 446.65a (b)(1). The potency of the drug is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of oxytetracycline that it is represented to contain.

(2) *Moisture.* If it contains oxytetracycline hydrochloride, proceed as directed in § 440.80a(b)(5)(i) of this chapter, and if it contains oxytetracycline proceed as directed in § 440.74a (b)(5) of this chapter.

§ 446.165c Oxytetracycline-nystatin capsules.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline-nystatin capsules are gelatin capsules containing oxytetracycline and nystatin with glucosamine hydrochloride and one or more suitable and harmless buffer substances, preservatives, binders, lubricants, and surfactants. Each capsule contains 250 milligrams of oxytetracycline and 250,000 units of nystatin. Its moisture content is not more than 7.5 percent. The oxytetracycline used conforms to the standards prescribed by § 446.65a(a)(1) (i), (iii), (vi), (vii), (viii), and (ix). The nystatin used conforms to the standards prescribed by § 449.50(a)(1) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline used in making the batch for potency, toxicity, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The nystatin used in making the batch for potency, toxicity, moisture, pH, and identity.

(*c*) The batch for oxytetracycline content, nystatin content, and moisture.

(ii) Samples required:

(*a*) The oxytetracycline used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) Nystatin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The batch: A minimum of 30 capsules.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Oxytetracycline content.* Proceed as directed in § 446.65a(b)(1) (i) or (ii), except prepare the sample as follows: Place a representative number of capsules in a glass blending jar containing 500 milliliters of 0.1*N* hydrochloric acid. Using a high-speed blender, blend for 3 to 5 minutes, and then make the proper estimated dilutions to the prescribed reference concentration in pH 4.5 buffer. Its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of oxytetracycline that it is represented to contain.

(ii) *Nystatin content.* Proceed as directed in § 449.50(b)(1) of this chapter, except prepare the sample as follows: Place a representative number of capsules in a blending jar with sufficient dimethyl formamide to give a stock solution of convenient concentration. Blend for 2 minutes in a high-speed

blender. Dilute an aliquot of the blended solution in sufficient dimethyl formamide to give a nystatin concentration of 400 units per milliliter (estimated) if the plate assay method is used, or 160 units per milliliter (estimated) if the turbidimetric assay method is used. Further dilute with 10 percent potassium phosphate buffer, pH 6.0, to 20 units per milliliter (estimated) if the plate assay method is used, or with distilled water to 16 units per milliliter (estimated) if the turbidimetric assay method is used. Its content of nystatin is satisfactory if it contains not less than 90 percent and not more than 135 percent of the number of units of nystatin it is represented to contain.

(2) *Moisture.* Proceed as directed in § 440.74a(b) (5) of this chapter.

NOTE: § 446.165c (formerly § 148n.10) was revoked at 34 FR 18161, Nov. 13, 1969. The effective date of the revocation was stayed at 34 FR 20427, Dec. 31, 1969, and further postponed at 35 FR 5811, Apr. 9, 1970.

§ 446.165d Oxytetracycline for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline for oral suspension is oxytetracycline, with one or more suitable and harmless buffer substances, preservatives, diluents, colorings, and flavorings. When prepared as directed in the labeling, each milliliter contains 50 milligrams of oxytetracycline. Its moisture content is not more than 2 percent. When reconstituted as directed in its labeling, its pH is not less than 5.5 and not more than 7.5. The oxytetracycline used conforms to the standards prescribed by § 446.65a(a) (1) (i), (iii), (vi), (vii), (viii), and (ix). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline used in making the batch for potency, toxicity, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency, moisture and pH.

(ii) Samples required:

(*a*) Oxytetracycline used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 6 immediate containers.

(c) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 446.65a(b) (1) (i) or (ii), except prepare the sample as follows: Reconstitute as directed in the labeling. Transfer an appropriate aliquot (usually from 1.0 milliliter to 5.0 milliliters) to a 100-milliliter volumetric flask, and make to mark with 0.1*N* hydrochloric acid. Withdraw an aliquot from the volumetric flask, and using pH 4.5 buffer solution dilute to the prescribed reference concentration. Its content of oxytetracycline is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of oxytetracycline that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 440.80a(b) (5) (i) of this chapter.

(3) *pH.* Reconstitute as directed in the labeling and proceed as directed in § 440.80a(b) (5) (ii) of this chapter.

§ 446.165e Oxytetracycline-nystatin for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline-nystatin for oral suspension is oxytetracycline and nystatin, with glucosamine hydrochloride and one or more suitable and harmless buffer substances, preservatives, diluents, colorings, and flavorings. When reconstituted as directed in the labeling, each milliliter shall contain 25 milligrams of oxytetracycline and 25,000 units of nystatin. Its moisture content is not more than 2 percent. When reconstituted as directed in the labeling, its pH is not less than 4.5 and not more than 7.5. The oxytetracycline used conforms to the standards prescribed by § 446.65a(a) (1) (i), (iii), (vi), (vii), (viii), and (ix). The nystatin used conforms to the standards prescribed by § 449.50(a) (1) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline used in making the batch for potency, toxicity, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The nystatin used in making the batch for potency, toxicity, moisture, pH, and identity.

(*c*) The batch for oxytetracycline content, nystatin content, moisture, and pH.

(ii) Samples required:

(*a*) The oxytetracycline used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The nystatin used in making the batch: 10 packages, each containing 300 milligrams.

(*c*) The batch: A minimum of 7 immediate containers.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Oxytetracycline content.* Proceed as directed in § 446.65a(b) (1) (i) or (ii), except prepare the sample as follows: Reconstitute the powder as directed in the labeling of the drug. Transfer an appropriate aliquot (usually from 1.0 milliliter to 5.0 milliliters) to a 100-milliliter volumetric flask, and make to mark with 0.1*N* hydrochloric acid. Withdraw an aliquot from the volumetric flask, and using pH 4.5 buffer, dilute to the prescribed reference concentration. Its content of oxytetracycline is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of oxytetracycline that it is represented to contain.

(ii) *Nystatin content.* Proceed as directed in § 449.50(b) (1) of this chapter, except prepare the sample as follows: Reconstitute the powder as directed in the labeling of the drug. Transfer an appropriate aliquot (usually from 1.0 milliliter to 5.0 milliliters) to a blending jar containing 160 milliliters of dimethyl formamide. Blend for 2 minutes in a high-speed blender and then dilute an aliquot with sufficient dimethyl formamide to give a nystatin concentration of 400 units per milliliter (estimated) if the plate assay method is used, or 160 units per milliliter (estimated) if the turbidimetric assay method is used. Further dilute with 10 percent potassium phosphate buffer, pH 6.0, to 20 units per milliliter (estimated) if the plate assay method is used, or with distilled water to 16 units per milliliter (estimated) if the turbidimetric assay method is used. Its content of nystatin is satisfactory if it contains not less than 90 percent and not more than 135 percent of the number of units it is represented to contain.

(2) *Moisture.* Proceed as directed in § 440.74a(b) (5) of this chapter.

(3) *pH.* Reconstitute as directed in the labeling and proceed as directed in § 440.80a(b) (5) (ii) of this chapter.

NOTE: § 446.165e (formerly § 148n.9) was revoked at 34 FR 18161, Nov. 13, 1969. The effective date of the revocation was stayed at 34 FR 20427, Dec. 31, 1969, and further postponed at 35 FR 5811, Apr. 9, 1970.

§ 446.166 Calcium oxytetracycline sirup.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Calcium oxytetracycline sirup is sirup that contains calcium oxytetracycline, with one or more suitable and harmless buffer substances, suspending and stabilizing agents, flavorings, colorings, solvents, and preservatives, suspended in a suitable and harmless vehicle. It contains *N*-acetyl glucosamine. Each milliliter contains a quantity of calcium oxytetracycline equivalent to either 25 milligrams or 100 milligrams of oxytetracycline. Its pH is not less than 5.0 and not more than 8.0. The calcium oxytetracycline used conforms to the standards prescribed by § 446.66(a) (1) (i), (ii), (iv), (v), and (vi). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 431.1 of

this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) Calcium oxytetracycline used in making the batch for potency, toxicity, pH, calcium content, identity, and crystallinity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The calcium oxytetracycline used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 446.65a (b) (1) (i) or (ii), except prepare the sample as follows: Transfer 1 milliliter of the sample to a 500-milliliter volumetric flask containing 100 milliliters of 0.1*N* hydrochloric acid. Dilute to volume with water and mix well. Transfer the suspension to a glass blending jar and blend for 5 minutes in a high-speed blender. Dilute an aliquot to the prescribed reference concentration, using 0.1*M* potassium phosphate buffer, pH 4.5. Its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of oxytetracycline that it is represented to contain.

(2) *pH.* Using the undiluted sample, proceed as directed in § 440.80a(b) (5) (ii) of this chapter.

§ 446.167 Oxytetracycline hydrochloride soluble tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride soluble tablets are composed of oxytetracycline hydrochloride with one or more suitable and harmless diluents and binders. Each tablet contains 50 milligrams of oxytetracycline. The moisture content is not more than 2.0 percent. The oxytetracycline hydrochloride used conforms to the standards prescribed by § 446.67a(a) (1) (i), (iii), (vi), (vii), (viii), and (ix). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline hydrochloride used in making the batch for potency, toxicity, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 tablets.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 446.65a (b) (1) (i) or (ii), except prepare the sample as follows: Place a representative number of tablets in a glass blending jar containing 500 milliliters of 0.1*N* hydrochloric acid. Using a high-speed blender, blend for 3 minutes and then make the proper estimated dilutions in 0.1*M* potassium phosphate buffer, pH 4.5. The potency is satisfactory if the tablets contain not less than 90 percent and not more than 115 percent of the number of milligrams of oxytetracycline they are represented to contain.

(2) *Moisture.* Proceed as directed in § 440.80a(b) (5) (i) of this chapter.

§ 446.180 Tetracycline oral dosage forms.

§ 446.180a Tetracycline-nystatin oral suspension; tetracycline phosphate complex-nystatin oral suspension (tetracycline phosphate complex-nystatin oral drops).

(a) *Requirements for certification.* Tetracycline-nystatin oral suspension and tetracycline phosphate complex-nystatin oral suspension are suspensions that conform to all the requirements and procedures prescribed by § 446.181c(a) for tetracycline oral suspension or tetracycline phosphate complex oral suspension, except that:

(1) Each milliliter contains not less than 25,000 units of nystatin. The nystatin used conforms to the standards prescribed by § 446.181b(a) (1).

(2) [Reserved]

(3) In addition to complying with the requirements of § 446.181c(a) (4), a person who requests certification of a batch shall submit with his request a statement showing the batch mark and (unless they were previously submitted) the results and the date of the latest tests and assays of the nystatin used in making the batch for potency, toxicity, pH, moisture, and identity. He shall also submit in connection with his request a sample consisting of not less than six immediate containers of the batch and (unless it was previously submitted) a sample consisting of 10 packages, each containing approximately equal portions of not less than 300 milligrams of the nystatin used in making the batch.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Tetracycline or tetracycline phosphate complex content.* Proceed as directed in § 446.181c(b) (1). Its content of tetracycline or tetracycline phosphate complex is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(ii) *Nystatin content.* Use 5 milliliters of the sample and proceed as directed in § 446.181b(b) (1) (i) (*b*). Its content of nystatin is satisfactory if it contains not less than 85 percent of the number of units that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 436.500(c) of this chapter.

NOTE: § 446.180a (a) and (b) (formerly § 146c.229 and § 141c.229, respectively) were revoked at 34 FR 18161, Nov. 13, 1969. The effective date of the revocation was stayed at 34 FR 20427, Dec. 31, 1969, and further postponed at 35 F.R. 5811, Apr. 9, 1970.

§ 446.180b Tetracycline-nystatin for oral suspension.

(a) *Requirements for certification.* Tetracycline-nystatin for oral suspension conforms to all requirements and procedures prescribed by § 446.510b(a) for tetracycline powder intended for use by man, except that:

(1) When reconstituted as directed in the labeling, each milliliter shall contain not less than 25 milligrams of tetracycline and not less than 25,000 units of nystatin. The nystatin used conforms to the standards prescribed by § 446.181b (a) (1).

(2) The pH of the suspension when reconstituted as directed in its labeling, is not less than 4.5 and not more than 5.5.

(3) It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(4) In addition to complying with the requirements of § 446.510b(a) (4), a person who requests certification of a batch shall submit with his request a statement showing the batch mark and (unless they were previously submitted) the results and the date of the latest tests and assays of the nystatin used in making the batch for potency, toxicity, pH, moisture, and identity. He shall also submit in connection with his request a sample consisting of not less than 6 immediate containers of the batch and (unless it was previously submitted) a sample consisting of 10 packages, each containing approximately equal portions of not less than 300 milligrams of the nystatin used in making the batch.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Tetracycline content.* Reconstitute the powder as directed in the labeling of the drug. Transfer an appropriate aliquot (usually from 1.0 milliliter to 5.0 milliliters) to a 100-milliliter volumetric flask and make to mark with 0.1*N* HCl. Withdraw an aliquot from the volumetric flask and dilute to a concentration of 0.24 microgram per milliliter (estimated) using *M*/10 phosphate buffer pH 4.5, and proceed as directed in § 446.81a(b) (1). Its content of tetracycline is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(ii) *Nystatin content.* Reconstitute the powder as directed in the labeling of the drug. Transfer an appropriate aliquot (usually from 1.0 milliliter to 5.0 milliliters) to a blending jar containing 150 milliliters of dimethyl formamide. Blend for 2 minutes in a high-speed blender and then dilute an aliquot with sufficient dimethyl formamide to give a concentration of 400 units per milliliter (estimated). Further dilute this solution

with 10 percent phosphate buffer, pH 6.0, to give a concentration of 20 units per milliliter (estimated). Proceed as directed in § 446.181b(b)(2). Its content of nystatin is satisfactory if it contains not less than 85 percent of the number of units that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) or § 440.74a(b)(5) of this chapter.

(3) *pH.* Using the suspension prepared as directed in the labeling, proceed as directed in § 440.80a(b)(5)(ii) of this chapter.

NOTE: § 446.180b (a) and (b) (formerly § 146c.236 and § 141c.236 respectively) were revoked at 34 FR 18161, Nov. 13, 1969. The effective date of the revocation was stayed at 34 FR 20427, Dec. 31, 1969, and further postponed at 35 FR 5811, Apr. 9, 1970.

§ 446.181 Tetracycline hydrochloride oral dosage forms.

§ 446.181a Tetracycline hydrochloride-nystatin tablets.

(a) *Requirements for certification.* Tetracycline hydrochloride-nystatin tablets are tablets that conform to all requirements and are subject to all procedures prescribed by § 446.181b(a) for tetracycline hydrochloride-nystatin capsules, except that the expiration date is the date that is 24 months after the month during which the batch was certified. In addition to the requirements prescribed by § 446.181b(a), tablets not exceeding 15 millimeters in diameter, or not intended only for preparing solutions, shall disintegrate within 1 hour. A person who requests certification shall therefore also submit for disintegration-time studies, results of this test made by him and a sample of six tablets.

(b) *Tests and methods of assay*—(1) *Tablets*—(i) *Potency*—(a) *Tetracycline hydrochloride content.* Proceed as directed in § 446.181b(b)(1)(i)(a). Its content of tetracycline hydrochloride is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(b) *Nystatin content.* Proceed as directed in § 446.181b(b)(1)(i)(b). Its content of nystatin is satisfactory if it contains not less than 85 percent of the number of units that it is represented to contain.

(ii) *Moisture.* Proceed as directed in § 440.80a(b)(1) or § 440.74a(b)(5) of this chapter.

(iii) *Distintegration time.* Proceed as directed in § 440.180a(b)(3) of this chapter.

(2) *Nystatin used in making the tablets.* Proceed as directed in § 446.181b(b)(2).

NOTE: § 446.181a (a) and (b) (formerly § 146c.225 and § 141c.225 respectively) were revoked at 34 FR 18161, Nov. 13, 1969. The effective date of the revocation was stayed at 34 FR 20427, Dec. 31, 1969, and further postponed at 35 FR 5811, Apr. 9, 1970.

§ 446.181b Tetracycline hydrochloride-nystatin capsules; tetracycline phosphate complex-nystatin capsules.

(a) *Requirements for certification.* Tetracycline hydrochloride-nystatin capsules and tetracycline phosphate complex-nystatin capsules are capsules that conform to all the requirements and procedures prescribed by § 446.110b(a) for tetracycline hydrchloride capsules and tetracycline phosphate complex capsules except that:

(1) Each capsule contains not less than 100,000 units of nystatin. The nystatin used is produced by the growth of *Streptomyces noursei.* It is a white to yellow to light-tan powder. It is very slightly soluble in water, moderately soluble in methyl alcohol, butyl alcohol, or propyl alcohol. Its potency is not less than 2,000 units per milligram. It is nontoxic. Its pH in a 3-percent aqueous suspension is not less than 6.5 and not more than 8.0. Its moisture content is not more than 5 percent. It exhibits absorption maxima, within ±2 mμ, at 230 mμ, 291 mμ, 305 mμ, and 319 mμ when dissolved in methyl alcohol and the ratio of the absorption peak at 230±2 mμ and the middle of the shoulder at 279±2 mμ, A_{230}/A_{279}, is not less than 0.90 and not more than 1.25.

(2) If it is tetracycline phosphate complex-nystatin capsules, its expiration date shall be 18 months after the month during which the batch was certified, except that the date may be one of the longer dates permitted in § 446.110b(a) if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time such drug as prepared by him complies with the standards prescribed therefor by this section.

(3) In addition to complying with the requirements of § 446.110b(a)(4), a person who requests certification of a batch shall submit with his request a statement showing the batch mark and (unless they were previously submitted) the results and the date of the latest tests and assays of the nystatin used in making the batch for potency, toxicity, pH, moisture, and identity. He shall also submit in connection with his request (unless it was previously submitted) a sample consisting of 10 packages, each containing approximately equal portions of not less than 300 milligrams of the nystatin used in making the batch.

(b) *Tests and methods of assay*—(1) *Capsules*—(i) *Potency*—(a) *Tetracycline hydrochloride content; tetracycline phosphate complex content.* Proceed as directed in § 446.110b(a)(1) for tetracycline hydrochloride capsules and tetracycline phosphate complex capsules. Its potency is satisfactory if it contains the equivalent of not less than 85 percent of the number of milligrams of tetracycline hydrochloride that it is represented to contain.

(b) *Nystatin content.* Prepare the sample for assay by placing a representative number of capsules in a blending jar with sufficient dimethylformamide (previously adjusted to pH 6.5±0.5 with concentrated H_2SO_4) to give a stock solution of convenient concentration. Blend for 2 minutes in a high-speed blender. Assay as directed in paragraph (b)(2) of this section. Its content of nystatin is satisfactory if it contains not less than 85 percent of the number of units that it is represented to contain.

(c) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) of this chapter.

(2) *Nystatin used in making the capsules*—(i) *Potency*—(a) *Cylinders (cups).* Use cylinders described in § 440.80a(b)(1)(i) of this chapter.

(b) *Culture media.* Use ingredients that conform to the standards, if any, prescribed by the U.S.P. or N.F.

(1) Make nutrient agar for carrying the organism and for preparing the inoculated agar plates as follows:

Peptone	9.4 grams.
Yeast extract	4.7 grams.
Beef extract	2.4 grams.
Sodium chloride	10.0 grams.
Dextrose	10.0 grams.
Agar	23.5 grams.
Distilled water to make	1,000 milliliters.

pH 6.0 to 6.2 after sterilization.

(2) Make nutrient broth for preparing an inoculum of the test organism:

Peptone	10 grams.
Dextrose	20 grams.
Distilled water to make	1,000 milliliters.

pH 6.6 to 6.7 after sterilization.

In lieu of preparing the media from the individual ingredients as specified, they may be prepared from a dehydrated mixture which, when reconstituted with distilled water, has the same composition as such media. Minor modifications of the specified individual ingredients are permissible if the resulting media possess growth-promoting properties at least equal to the media described.

(c) *Working standard.* Dry an appropriate amount of the working standard (obtained from the U.S.P. Reference Standards Committee, 46 Park Avenue, New York 16, New York) for 2 hours at 40° C. at a pressure of 5 millimicrons or less. Dissolve a weight of the dried standard in sufficient dimethylformamide to give a stock solution of convenient concentration (usually 1,000 units to 5,000 units per milliliter). This stock solution should be prepared simultaneously with the samples to be tested and should be used for 1 day only.

(d) *Preparation of sample.* Dissolve the sample to be tested in sufficient dimethylformamide to give a nystatin concentration of 400 units per milliliter (estimated). Further dilute with 10 percent potassium phosphate buffer, pH 6.0, to 20 units per milliliter (estimated).

(e) The test organism is Saccharomyces cerevisiae (ATCC 9763 or ATCC 2601[1] (no vitamin requirement)), which is maintained on slants of agar described under paragraph (b)(2)(i)(b)(1) of this section and transferred once a week. After transfer, the culture is incubated at 37° C. for 24 hours and then kept refrigerated. Prepare the organism suspension by either of the following methods:

(1) Inoculate 100 milliliters of the broth medium described under paragraph (b)(2)(i)(b)(2) of this section with a loopful of growth from the agar slant. Incubate the broth for 16 to 18 hours at 37° C. This broth culture may be used for several weeks if kept refrigerated.

(2) Wash the organism from the agar slant with 3 milliliters of sterile physiological saline solution onto a large agar

surface such as that provided by a Roux bottle containing 300 milliliters of the agar described in paragraph (b) (2) (i) (*b*) (*1*) of this section. Spread the suspension of organisms over the entire agar surface with the aid of sterile glass beads. Incubate for 24 hours at 37° C. and then wash the resulting growth from the agar surface with about 30 milliliters of sterile sodium chloride solution. This suspension may be used for several weeks if kept refrigerated. Run test plates to determine the quantity of this broth culture (usually about 2.5 milliliters) or saline suspension (usually about 0.1 milliliter) that should be added to each 100 milliliters of agar to give clear, sharp inhibitory zones of appropriate size.

(*f*) *Preparation of plates.* Melt a sufficient amount of the agar described in paragraph (b) (2) (i) (*b*) (*1*) of this section, cool to 48° C., add the proper amount of the test organism as described above, and mix thoroughly. Add 8 milliliters of this inoculated agar to each Perti dish (100 millimeters x 22 millimeters, with flat bottom). Distribute the agar evenly in the plates, cover with porcelain covers glazed on the outside, and allow to harden. After the agar has hardened, place 6 cylinders on the agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius.

(*g*) Standard curve. Dilute aliquots of the standard stock solution with dimethylformamide to give concentrations of 256, 320, 400, 500, and 624 units per milliliter. Dilute these solutions with 10 percent potassium phosphate buffer, pH 6.0, to make concentrations of 12.8, 16.0, 20.0, 25.0, and 31.2 units per milliliter, respectively. A total of 12 plates is used in the preparation of the standard curve, three plates for each solution, except the 20 units per milliliter solution. The latter concentration is used as the reference point and is included on each plate. On each of the three plates fill three cylinders with the 20 units per milliliter standard and the other three cylinders with the concentration of the standard concentration under test. Thus, there will be 36 twenty-unit determinations and nine determinations for each of the other concentrations on the curve. Incubate the plates for 16 to 18 hours at 37° C. and measure the diameter of each zone of inhibition. Average the readings of the 20 units per milliliter concentration and the readings of the concentration tested for each set of three plates, and average also all 36 readings of the 20 units per milliliter concentration. The average of the 36 readings of the 20 units per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 20 units per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 16 units per milliliter concentration the average of the 36 readings of the 20 units per milliliter concentration is 16.0 millimeters and the average of the 20 units per milliliter concentration of this set of three plates is 15.8 millimeters, the correction is +0.2 millimeter. If the average reading of the 15 units per milliliter concentration of those same three plates is 15.0 millimeters, the corrected value is then 15.2 millimeters. Plot these corrected values, including the average of the 20 units per milliliter concentration on two-cycle semilog paper, using the concentration in micrograms per milliliter as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points.

(*h*) *Assay.* Use 3 plates for each sample. Fill three cylinders on each plate with the 20 units per milliliter standard and three cylinders with the 20 units per milliliter (estimated) sample, alternating standard and sample. Incubate the plates for 16 to 18 hours at 37° C. and then measure the diameter of each zone of inhibition. To estimate the potency of the sample, average the zone readings of the standard and the zone readings of the sample on the three plates used. If the sample gives a larger zone size than the average of the standard, add the difference between them to the 20 units per milliliter zone on the standard curve. If the average value is lower than the standard value, subtract the difference between them from the 20 units per milliliter value on the curve. From the curves read the potencies corresponding to these corrected values of zone sizes.

(*i*) *Turbidimetric assay—(1) Test culture and media.* Maintain the test organism *Saccharomyces cerevisiae* (ATCC 9763)[1] as described in paragraph (b) (2) (i) (*e*) of this section. For use in the assay, prepare an organism suspension as directed in paragraph (b) (2) (i) (*e*) (*1*) of this section. Adjust this stock suspension, so that a 1:10 dilution in the nutrient broth will have an absorption of 0.350 at a wavelength of 650 mμ, using a suitable photoelectric colorimeter and an 18-millimeter diameter test tube as an absorption cell. Prepare the inoculated broth by adding from 3 milliliters to 5 milliliters of that adjusted suspension (not the 1: 10 dilution) to each liter of nutrient broth, prepared as directed in paragraph (b) (2) (i) (*b*) (2) of this section, needed for the test.

(*2*) *Working standard and standard solutions.* Prepare the standard stock solution as directed in paragraph (b) (2) (i) (*c*) of this section. Make dilutions of the stock solution with dimethylformamide to obtain concentrations of 121, 139, 160, 184, and 212 units per milliliter. These solutions are then diluted 1:10 with sterile distilled water to obtain concentrations of 12.1, 13.9, 16.0, 18.4, and 21.2 units per milliliter. Add 1.0 milliliter of each such concentration to each of six sterile 20 millimeters x 150 millimeters test tubes. Maintain the sterility of the tubes.

(*3*) *Preparation of sample.* Dissolve the sample to be tested in sufficient dimethylformamide to give a stock solution of convenient concentration. Dilute the stock solution with dimethylformamide to obtain a concentration of 160 units per milliliter (estimated). Further dilute this solution 1:10 with sterile distilled water to obtain a concentration of 16 units per milliliter (estimated). Add 1.0 milliliter of this dilution to each of six sterile 20 millimeters x 150 millimeters test tubes.

(*4*) *Procedure.* To each of the 20 millimeters x 150 millimeters test tubes prepared in paragraph (b) (2) (i) (*i*) (2) and (*3*) of this section, add 9.0 milliliters of the inoculated nutrient broth described in paragraph (b) (2) (i) (*i*) (*1*) of this section, and incubate at 26° C. for 16 hours to 18 hours. After incubation, add 0.5 milliliter of a 12 percent formaldehyde solution to each tube and read the absorbance values in a suitable photoelectric colorimeter, using a wavelength of 530 mμ. Set the instrument at zero absorbance with clear uninoculated broth prepared as described in paragraph (b) (2) (i) (*b*) (2) of this section.

(*5*) *Estimation of potency.* Plot the average values for each concentration of the standard on semilog graph paper with absorbance values on the arithmetic scale and nystatin concentrations on the logarithmic scale. Construct the best straightline through the points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5}$$

$$H=\frac{3e+2d+c-a,}{5}$$

where

L=absorbance value for the lowest concentration of the standard curve.

H=absorbance value for the highest concentration of the standard curve.

a, b, c, d, e=average absorbance values for each concentration of the standard curve.

Plot the values obtained for L and H and connect the points with a straightline. Average the absorbance values for the sample and read the nystatin concentration from the standard curve. Multiply the concentration by appropriate dilution factors to obtain the nystatin content of the sample. Its content of nystatin is satisfactory if it is not less than 85 percent of the number of units that it is represented to contain.

(ii) *Toxicity.* Inject intraperitoneally each of 5 mice, within the weight range of 18 grams to 25 grams, with 0.5 milliliter of a suspension of the drug containing 1,200 units per milliliter in a 0.5 percent acacia solution. If no animal dies within 48 hours, the sample is nontoxic. If one or more animals die within 48 hours, repeat the test, using for each test five or more previously unused mice weighing 20 grams (±0.5 gram) each; if the total deaths within 48 hours is no greater than 10 percent of the total number of animals tested, including the original test, the sample is nontoxic.

(iii) *Moisture.* Proceed as directed in § 440.80a(b) (5) (i) of this chapter.

(iv) *pH.* Proceed as directed in § 440.80a(b) (5) (ii) of this chapter, using a 3-percent aqueous suspension of the drug.

(v) *Identity.* Weigh approximately 20 milligrams of the sample in a 100-milliliter glass-stoppered volumetric flask,

add about 75 milliliters absolute methyl alcohol, and shake mechanically for 30 minutes. Dilute to volume with methyl alcohol. Transfer 10.0 milliliters of this solution to a 100-milliliter volumetric flask and dilute to volume with methyl alcohol. Within 2 hours, determine the absorption peak at 230 mμ, 291 mμ, 305 mμ, and 319 mμ and the shoulder at 279±2 mμ, using a suitable ultraviolet spectrophotometer and quartz cells. Set the instrument to 100 percent transmission with absolute methyl alcohol. If a recording spectrophotometer is used, record the ultraviolet absorption spectrum from 220 mμ to 320 mμ. If a nonrecording spectrophotometer is used, the exact positions of the peaks and shoulder should be determined for the particular instrument used. The ratio of the two absorbancies (A_{305}/A_{291}) should be not less than 0.90 and not more than 1.25.

NOTE: § 446.181b (a) and (b) (formerly § 146c.224 and § 141c.224 respectively) were revoked at 34 FR 18161, Nov. 13, 1969. The effective date of the revocation was stayed at 34 FR 20427, Dec. 31, 1969, and further postponed at 35 FR 5811, Apr. 9, 1970.

§ 446.181c Tetracycline hydrochloride oral suspension (tetracycline hydrochloride homogenized mixture); tetracycline phosphate complex oral suspension (tetracycline phosphate complex oral drops); tetracycline hydrochloride oral solution; tetracycline calcium oral suspension; tetracycline oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline hydrochloride oral suspension, tetracycline hydrochloride oral solution, and tetracycline calcium oral suspension are tetracycline hydrochloride of tetracycline calcium prepared from tetracycline hydrochloride. Tetracycline oral suspension and tetracycline phosphate complex oral suspension are prepared from tetracycline or tetracycline phosphate complex. Each drug contains one or more suitable and harmless suspending and dispersing agents (unless it is tetracycline hydrochloride oral solution), with or without one or more suitable and harmless colorings, flavorings, buffer substances, and preservatives, suspended or dissolved in a suitable and harmless vehicle. Each milliliter shall contain not less than the equivalent of 25 milligrams of tetracycline hydrochloride. Its moisture content is not more than 2 percent if it is tetracycline hydrochloride oral suspension, tetracycline phosphate complex oral suspension, or tetracycline oral suspension and not more than 7 percent if it is tetracycline hydrochloride oral solution. Its pH is not less than 2.0 and not more than 3.5 if it is tetracycline hydrochloride oral solution and not less than 7.0 and not more than 8.0 if it is tetracycline calcium oral suspension. The tetracycline hydrochloride used conforms to the standards prescribed by § 446.81a (a) (1), except § 446.81a(a) (1) (ii), (iv), and (v). The tetracycline phosphate complex used conforms to the standards prescribed by § 446.82(a) (1). The tetracycline used conforms to the standards prescribed by § 446.80(a) (1). Each other substance used, if its name is recognized in the U. S. P. or N. F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* The immediate container shall be a tight container as defined by the U. S. P., and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused that are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on the outside wrapper or container and the immediate container, as hereinafter indicated, the statement "Expiration date _____", the blank being filled in with the date that is 18 months after the month during which the batch was certified, except that the blank may be filled in with the date that is 24 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time such drug as prepared by him complies with the standards prescribed by paragraph (a) (1) of this section.

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless it was previously submitted) the date on which the latest assay of the tetracycline hydrochloride, tetracycline, or tetracycline phosphate complex used in making such batch was completed, the potency per milliliter of the batch, the date on which the latest assay of the drug comprising such batch was completed, the quantity of each ingredient used in making the batch, and a statement that each such ingredient conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided in paragraph (a) (4) (iv) of this section, each person shall submit in connection with his request results of the tests and assays listed after each of the following made by him on an accurately representative sample of:

(*a*) The batch: Average potency per milliliter, average moisture if it is tetracycline hydrochloride oral suspension, tetracycline oral suspension, tetracycline phosphate complex oral suspension, or tetracycline hydrochloride oral solution, and pH if it is tetracycline hydrochloride oral solution or tetracycline calcium oral suspension.

(*b*) The tetracycline hydrochloride, tetracycline, or tetracycline phosphate complex used in making the batch: Potency, toxicity, moisture, pH, crystallinity, and absorptivity, and if it is tetracycline phosphate complex, identity.

(iii) Except as otherwise provided by paragraph (a) (4) (iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch: 1 immediate container for each 5,000 immediate containers in the batch, but in no case less than 5 immediate containers, collected by taking single immediate containers at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(*b*) The tetracycline hydrochloride, tetracycline, or tetracycline phosphate complex used in making the batch: 10 packages, each containing approximately equal portions of not less than 60 milligrams, and if it is tetracycline phosphate complex, one additional package containing approximately 1 gram. Each such package shall be packaged in accordance with the requirements of § 446.10a(a) (2).

(*c*) In the case of an initial request for certification, each other ingredient used in making the batch: 1 package of each containing approximately 5.0 grams.

(iv) No result referred to in paragraph (a) (4) (ii) (*b*) of this section, and no sample referred to in paragraph (a) (4) (iii) (*b*) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay*—(1) *Potency.* Transfer 1 milliliter of the well-shaken suspension to a suitable high-speed blender containing 500 milliliters of 0.1 *N* HCl. Blend for 5 minutes and proceed as directed in § 446.81a(b) (1). Its potency is satisfactory if it contains not less than 85 percent of the number of milligrams, or the equivalent number of milligrams, of tetracycline hydrochloride per milliliter that it is represented to contain.

(2) *Moisture.* If it is tetracycline hydrochloride oral suspension, tetracycline oral suspension, tetracycline hydrochloride oral solution, or tetracycline phosphate complex oral suspension, proceed as directed in § 436.500 of this chapter.

(3) *pH.* If it is tetracycline hydrochloride oral solution or tetracycline calcium oral suspension, use the undiluted drug and proceed as directed in § 440.80a (b) (5) (ii) of this chapter.

Subpart C—Injectable Dosage Forms

§ 446.210 Sterile chlortetracycline hydrochloride.

The requirements for certification and the tests and methods of assay for sterile chlortetracycline hydrochloride packaged for dispensing are described in § 446.10a.

§ 446.220 Doxycycline hyclate for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Doxycycline hyclate for injection is a dry mixture of doxycycline hyclate and a buffer substance. Its potency is satisfactory if it is not less than 90 percent and not more than 120

percent of the number of milligrams of doxycycline that it is represented to contain. It is sterile. It is nonpyrogenic. It passes the safety test. It contains no histamine nor histamine-like substances. Its loss on drying is not more than 2.0 percent. Its pH when reconstituted as directed in the labeling is not less than 1.8 and not more than 3.3. It passes the identity test for the presence of the doxycycline moiety. The doxycycline hyclate used conforms to the standards prescribed by § 446.20a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(3) *Requests for certification: samples.* In addition to complying with the requirements of § 431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The doxycycline hyclate used in making the batch for potency, moisture, pH, doxycycline content, identity, and crystallinity.

(*b*) The batch for potency, sterility, pyrogens, safety, histamine, loss on drying, pH, and identity.

(ii) Samples required:

(*a*) The doxycycline hyclate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this subchapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents from each container if it is represented as a single-dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the solution thus obtained with sufficient 0.1*N* hydrochloric acid to give a stock solution of convenient concentration (containing not less than 150 micrograms of doxycycline in acid). Further dilute an aliquot with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 0.100 microgram of doxycycline per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this subchapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this subchapter, using a solution containing 7.5 milligrams of doxycycline per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this subchapter.

(5) *Histamine.* Proceed as directed in § 436.35 of this subchapter.

(6) *Loss on drying.* Proceed as directed in § 436.200(a) of this subchapter.

(7) *pH.* Proceed as directed in § 436.202 of this subchapter, using the drug reconstituted as directed in the labeling.

(8) *Identity.* Proceed as directed in § 436.308 of this subchapter, except prepare the standard and sample solutions as follows: Dissolve precise amounts of the doxycycline hyclate for injection and of the doxycycline working standard in methanol and further dilute each solution to a concentration of 1 milligram of doxycycline per milliliter. Prepare the sample-standard mixed solution by mixing equal volumes of the final concentration of the sample and standard solutions. The sample and standard must each produce a major, yellow fluorescent spot with the same R_f value and the sample-standard mixed solution must show no separation of major spots.

§ 446.260 Sterile minocycline hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile minocycline hydrochloride is a lyophilized powder of minocycline hydrochloride. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of minocycline that it is represented to contain. It is sterile. It is nonpyrogenic. It passes the safety test. It contains no histamine nor histamine-like substance. Its moisture content is not more than 3.0 percent. Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.0 and not more than 3.5. The minocycline hydrochloride used conforms to the standards prescribed by § 446.60(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The minocycline hydrochloride used in making the batch for potency, moisture, pH, minocycline content, identity, and crystallinity.

(*b*) The batch for potency, sterility, pyrogens, safety, histamine, moisture, and pH.

(ii) Samples required:

(*a*) The minocycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Using a suitable hypodermic needle and syringe, remove the withdrawable contents from each container represented as a single-dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, withdraw an accurately measured representative portion from each container. Dilute the sample thus obtained with sufficient 0.1*N* hydrochloric acid to give a stock solution of convenient concentration not less than 150 micrograms of minocycline per milliliter (estimated). Further dilute the stock solution with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 0.100 microgram of minocycline per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 5 milligrams per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this chapter.

(5) *Histamine.* Proceed as directed in § 436.35 of this chapter.

(6) *Moisture.* Proceed as directed in § 436.201 of this chapter, using the sample preparation described in paragraph (d)(4) of that section.

(7) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams of minocycline per milliliter.

§ 446.265 Oxytetracycline intramuscular solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline intramuscular solution is oxytetracycline, with or without one or more suitable and harmless buffer substances, anesthetics, preservatives, antioxidants, complexing agents, and solvents. Its potency is 50 milligrams or 125 milligrams of oxytetracycline per milliliter. It is sterile. It is nontoxic. It is nonpyrogenic. It contains no histamine nor histamine-like substances. Its pH is not less than 8.0 and not more than 9.0. The oxytetracycline used conforms to the standards prescribed by § 446.65a(a)(1) (i), (vi), (vii), (viii), and (ix). Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline used in making the batch for potency, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency, sterility, toxicity, pyrogens, histamine, and pH.

(ii) Samples required:

(*a*) Oxytetracycline used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 10 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(c) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 446.65a (b) (1) (i) or (ii), except prepare the sample as follows: Transfer a representative quantity of the sample equivalent to one dose to a 100-milliliter volumetric flask. Make to mark with 0.1*N* hydrochloric acid. Further dilute in pH 4.5 buffer solution to the prescribed reference concentration. Its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of oxytetracycline per milliliter that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e) (1) of that section.

(3) *Toxicity.* Proceed as directed in § 440.80a(b) (4) of this chapter, except use sterile distilled water as the diluent, and inject intraperitoneally 0.5 milliliter of a solution containing 2 milligrams of oxytetracycline per milliliter.

(4) *Pyrogens.* Proceed as directed in § 440.80a(b) (3) of this chapter, using as a test dose 1.0 milliliter per kilogram of a solution containing 5.0 milligrams of oxytetracycline per milliliter of sterile, pyrogen-free distilled water.

(5) *Histamine.* Proceed as directed in § 444.70a(b) (5) of this chapter, using as a test dose 0.6 milliliter per kilogram of a solution containing 5 milligrams of oxytetracycline per milliliter in sterile distilled water.

(6) *pH.* Using the undiluted solution, proceed as directed in § 440.80a(b) (5) (ii) of this chapter.

§ 446.267 Oxytetracycline hydrochloride for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride for injection is a dry mixture of oxytetracycline hydrochloride and a suitable buffer substance. Each vial contains 250 milligrams, 500 milligrams, 1.0 gram, or 2.5 grams of oxytetracycline. It is sterile. It is nontoxic. It is nonpyrogenic. It contains no histamine nor histamine-like substances. Its moisture content is not more than 3.0 percent. The pH of an aqueous solution containing 25 milligrams of oxytetracycline per milliliter is not less than 1.8 and not more than 2.8. The oxytetracycline hydrochloride used conforms to the standards prescribed by § 446.67a(a) (1) (i), (vi), (vii), (viii), and (ix). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The oxytetracycline hydrochloride used in making the batch for potency, moisture, pH, absorptivity, crystallinity, and identity.

(b) The batch for potency, sterility, toxicity, pyrogens, histamine, moisture, and pH.

(ii) Samples required:

(a) Oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 10 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(c) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 446.65a (b) (1) (i) or (ii), except reconstitute the product as directed in the labeling. Using a suitable syringe and hypodermic needle, promptly withdraw a suitable, measured aliquot of the solution and prepare an appropriate stock solution by prompt dilution with 0.1*N* hydrochloric acid. Dilute further in 0.1*M* potassium phosphate buffer, pH 4.5, to the prescribed reference concentration. The potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of oxytetracycline that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e) (1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Toxicity.* Proceed as directed in § 440.80a(b) (4) of this chapter, using as a test dose 0.5 milliliter of sterile distilled water solution containing 2.0 milligrams per milliliter.

(4) *Pyrogens.* Proceed as directed in § 440.80a(b) (3) of this chapter, using as a test dose 1.0 milliliter per kilogram of a sterile, pyrogen-free, distilled water solution containing 5.0 milligrams per milliliter.

(5) *Histamine.* Proceed as directed in § 444.70a(b) (5) of this chapter, using as a test dose 0.6 milliliter per kilogram of a solution containing 5 milligrams per milliliter prepared with the diluent recommended by the manufacturer in the labeling for the drug.

(6) *Moisture.* Proceed as directed in § 440.80a(b) (5) (i) of this chapter.

(7) *pH.* Proceed as directed in § 440.80a(b) (5) (ii) of this chapter, using an aqueous solution containing 25 milligrams of oxytetracycline per milliliter.

§ 446.275 Rolitetracycline injectable dosage forms.

§ 446.275a Rolitetracycline for intravenous use.

(a) *Requirements for certification*—(1) *Standard of identity, strength, quality, and purity.* Rolitetracycline for intravenous use is a dry mixture of rolitetracycline and one or more suitable buffer substances. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of rolitetracycline that it is represented to contain. It is sterile. It is nonpyrogenic. It passes the safety test. It contains no histamine nor histamine-like substances. Its loss on drying is not more than 5 percent. When reconstituted as directed in the labeling, its pH is not less than 3.0 and not more than 4.5. The rolitetracycline used conforms to the standards prescribed by § 446.75a(a) (1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The rolitetracycline used in making the batch for potency, moisture, pH, crystallinity, absorptivity, and identity.

(b) The batch for potency, sterility, pyrogens, safety, histamine, loss on drying, and pH.

(ii) Samples required:

(a) The rolitetracycline used in making the batch: 10 packages, each containing approximately 500 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 10 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this subchapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Add sufficient methyl alcohol to give a solution containing 1 milligram of rolitetracycline per milliliter (estimated). Further dilute an aliquot of this solution with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 0.25 microgram of rolitetracycline per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this subchapter, using the method described in paragraph (e) (1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this subchapter, using a solution containing 5.0 milligrams of rolitetracycline per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this subchapter.

(5) *Histamine.* Proceed as directed in § 436.35 of this subchapter.

(6) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(7) *pH.* Proceed as directed in § 436.202 of this subchapter, using a solution prepared as directed in the labeling.

§ 446.275 Rolitetracycline for intramuscular use.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Rolitetracycline for intramuscular use is a dry mixture of rolitetracycline and one or more suitable buffer substances and anesthetic agents. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of rolitetracycline that it is represented to contain. It is sterile. It is nonpyrogenic. Its loss on drying is not more than 5 percent. When reconstituted as directed in the labeling, its pH is not less than 3.0 and not more than 4.5. The rolitetracycline used conforms to the standards prescribed by § 446.75a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The rolitetracycline used in making the batch for potency, safety, histamine, moisture, pH, crystallinity, absorptivity, an identity.

(*b*) The batch for potency, sterility, pyrogens, loss on drying, and pH.

(ii) Samples required:

(*a*) The rolitetracycline used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this subchapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Add sufficient methyl alcohol to give a solution containing 1 milligram of rolitetracycline per milliliter (estimated). Further dilute an aliquot of this solution with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 0.25 microgram of rolitetracycline per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this subchapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this subchapter, using a solution containing 5.0 milligrams of rolitetracycline per milliliter.

(4) *Loss on drying.* Proceed as directed in § 436.200(b) of this subchapter.

(5) *pH.* Proceed as directed in § 436.202 of this subchapter, using a solution prepared as directed in the labeling.

§ 446.276 Rolitetracycline nitrate injectable dosage forms.

§ 446.276a Rolitetracycline nitrate for intravenous use.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Rolitetracycline nitrate for intravenous use is a dry mixture of rolitetracycline nitrate and one or more suitable buffer substances. Its potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of rolitetracycline that it is represented to contain. It is sterile. It is nonpyrogenic. It passes the safety test. It contains no histamine nor histamine-like substances. Its loss on drying is not more than 5 percent. When reconstituted as directed in the labeling, its pH is not less than 2.5 nor more than 4.0. The rolitetracycline nitrate used conforms to the standards prescribed by § 446.76a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(3) *Requests for certification; samples.* In addition to complying with requirements of § 431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The rolitetracycline nitrate used in making the batch for potency, moisture, pH, crystallinity, absorptivity, and identity.

(*b*) The batch for potency, sterility, pyrogens, safety, histamine, loss on drying, and pH.

(ii) Samples required:

(*a*) The rolitetracycline nitrate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this subchapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Add sufficient methyl alcohol to give a solution containing 1 milligram of rolitetracycline per milliliter (estimated). Further dilute an aliquot of this solution with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 0.25 microgram of rolitetracycline per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this subchapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this subchapter, using a solution containing 5.0 milligrams of rolitetracycline per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this chapter.

(5) *Histamine.* Proceed as directed in § 436.35 of this chapter.

(6) *Loss on drying.* Proceed as directed in § 436.200(b) of this subchapter.

(7) *pH.* Proceed as directed in § 436.202 of this subchapter, using a solution prepared as directed in the labeling.

§ 446.276b Rolitetracycline nitrate for intramuscular use.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Rolitetracycline nitrate for intramuscular use is a dry mixture of rolitetracycline nitrate, one or more suitable buffer substances, and lidocaine hydrochloride. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of rolitetracycline that it is represented to contain. It is sterile. It is nonpyrogenic. Its loss on drying is not more than 5 percent. When reconstituted as directed in the labeling, its pH is not less than 2.5 nor more than 4.0. The rolitetracycline nitrate used conforms to the standards prescribed by § 446.76a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The rolitetracycline nitrate used in making the batch for potency, safety, histamine, moisture, pH, crystallinity, absorptivity, and identity.

(*b*) The batch for potency, sterility, pyrogens, loss on drying, and pH.

(ii) Samples required:

(*a*) The rolitetracycline nitrate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this subchapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Add sufficient methyl alcohol to give a solution containing 1 milligram of rolitetracycline per milliliter (estimated). Further dilute an aliquot of this solution with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 0.25 microgram of rolitetracycline per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this subchapter, using the

method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this subchapter, using a solution containing 5.0 milligrams of rolitetracycline per milliliter.

(4) *Loss on drying.* Proceed as directed in § 436.200(b) of this subchapter.

(5) *pH.* Proceed as directed in § 436.202 of this subchapter, using a solution prepared as directed in the labeling.

§ 446.281 Tetracycline hydrochloride injectable dosage forms.

§ 446.281a Sterile tetracycline hydrochloride.

The requirements for certification and the tests and methods of assay for sterile tetracycline hydrochloride packaged for dispensing are described in § 446.81a.

§ 446.281b Tetracycline hydrochloride for intramuscular use; tetracycline phosphate complex for intramuscular use.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Tetracycline hydrochloride or tetracycline phosphate complex for intramuscular use is a dry mixture of tetracycline hydrochloride or tetracycline phosphate complex, magnesium chloride or magnesium ascorbate and one or more suitable buffer substances, with or without one or more suitable preservatives and anesthetic agents, and with or without one or more suitable solubilizers and stabilizers. It is sterile. It is nonpyrogenic. Its moisture content is not more than 5 percent. Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.0 and not more than 3.0. The tetracycline hydrochloride used conforms to the requirements of § 446.81a(a)(1). The tetracycline phosphate complex used conforms to the requirements of § 446.82(a)(1) and it contains no histamine or histamine-like substance. Each other substance used, if its name is recognized in the U. S. P. or N. F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* In all cases the immediate containers shall be tight containers as defined by the U. S. P., shall be sterile at the time of filling and closing, shall be so sealed that the contents cannot be used without destroying the seal, and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefore in applicable standards, except that minor changes so caused that are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded. The immediate containers shall be of colorless transparent glass, closed by a substance through which a hypodermic needle may be introduced and withdrawn without removing the closure or destroying its effectiveness. Each such container shall contain not less than the equivalent of 100 milligrams of tetracycline hydrochloride, and each may be packaged in combination with a container of a suitable and harmless diluent.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106 (b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the outside wrapper or container and the immediate container, the statement "Expiration date _____", the blank being filled in with the date that is 24 months (if it contains tetracycline phosphate complex) or 36 months (if it contains tetracycline hydrochloride) after the month during which the batch was certified, except that the blank may be filled in with the date that is 48 months or 60 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time such drug as prepared by him complies with the standards prescribed by paragraph (a)(1) of this section.

(ii) [Reserved]

(iii) On the circular or other labeling within or attached to the package, a statement of the conditions under which solutions prepared from the drug should be stored and the statement "Sterile solutions may be stored at room temperature for 24 hours without significant loss of potency".

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless it was previously submitted) the date on which the latest assay of the tetracycline hydrochloride or tetracycline phosphate complex used in making such batch was completed, the equivalent number of milligrams of tetracycline hydrochloride in each package, the date on which the latest assay of the drug comprising the batch was completed, the quantity of each other ingredient used in making the batch, and a statement that each such ingredient conforms to the requirements prescribed therefor by this section. If such batch or any part thereof is to be packaged with a diluent, such request shall also be accompanied by a statement that such diluent conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided by paragraph (a)(4)(v) of this section, such person shall submit in connection with his request the results of the tests and assays listed after each of the following, made by him on an accurately representative sample of:

(*a*) The batch: Potency, sterility, pyrogens, moisture, pH.

(*b*) The tetracycline hydrochloride or tetracycline phosphate complex used in making the batch: Potency, toxicity, histamine, absorptivity, crystallinity, and identity if it is tetracycline phosphate complex.

(iii) Except as otherwise provided by paragraph (a)(4)(v) of this section, if such batch is packaged for dispensing, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch:

(*1*) For all tests except sterility: One immediate container for each 5,000 immediate containers in the batch, but in no case less than 10 immediate containers.

Such samples shall be collected by taking single immediate containers at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(*2*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(*b*) The tetracycline hydrochloride or tetracycline phosphate complex used in making the batch: 10 packages, each containing approximately equal portions of not less than 250 milligrams, and if it is tetracycline phosphate complex, one additional package containing approximately 1 gram. Each such package shall be packaged in accordance with the requirements of § 446.10a(a)(2).

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: 1 package of each, containing approximately 5 grams.

(*d*) In case of an initial request for the certification of a batch which is to be packaged in combination with a diluent that is not recognized by the U. S. P. or when any change is made in the composition of such diluent: 5 packages of the diluent included in the combination.

(iv) If such batch is packaged for repacking, such person shall submit with his request a sample consisting of the following:

(*a*) For all tests except sterility: 10 packages, each containing approximately 0.25 gram.

(*b*) For sterility testing: 20 packages, each containing approximately 50 milligrams of tetracycline hydrochloride or tetracycline phosphate complex.

Each such package shall be packaged in accordance with the requirements of paragraph (a)(2) of this section.

(v) No result referred to in paragraph (a)(4)(ii)(*b*) of this section and no sample referred to in paragraph (a)(4)(iii)(*b*) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay—*(1) *Potency.* Reconstitute the sample as directed on the label or labeling. Using a suitable syringe, withdraw a one-dose aliquot and place in a 100-milliliter volumetric flask. Make to mark with 0.1 *N* HCl, and proceed as directed in § 446.81a(b)(1). Its potency is satisfactory if the immediate container contains not less than 90 percent of the number of milligrams of tetracycline hydrochloride or tetracycline phosphate complex that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except that if the product contains tetracycline hydrochloride use diluting fluid D in lieu of diluting fluid A, or if the product contains tetracycline

phosphate complex use 50 milligrams in lieu of 300 milligrams.

(3) *Pyrogens.* Proceed as directed in § 446.81a(b)(3).

(4) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) of this chapter.

(5) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(6) *Histamine in the tetracycline phosphate complex used in making the batch.* Proceed as directed in § 444.70a(b)(5) of this chapter, using as a test dose 0.6 milliliter of a solution containing 5 milligrams per milliliter prepared by dissolving 40 milligrams in 2.0 milliliters of 0.1 *N* HCl and diluting with the required amount of water.

Subpart D—Ophthalmic Dosage Forms

§ 446.310 Chlortetracycline hydrochloride ophthalmic dosage forms.

§ 446.310a Chlortetracycline ophthalmic (chlortetracycline hydrochloride ophthalmic); tetracycline hydrochloride ophthalmic.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Chlortetracycline ophthalmic and tetracycline hydrochloride opthalmic is crystalline chlortetracycline or crystalline tetracycline hydrochloride, with or without one or more suitable and harmless preservatives, buffer substances, and diluents. It is sterile. Its moisture content is not more than 5 percent. The chlortetracycline or tetracycline hydrochloride is of such quantity that when dissolved as directed the potency of such solution is not less than 1,000 micrograms per milliliter and maintains its labeled potency after it has been kept for 7 days at a temperature of 15° C. (59° F.). Such solution has a pH of not less than 7.0 and not more than 8.4. The chlortetracycline used conforms to the requirements of § 446.10a(a)(1) except paragraphs (a)(1)(ii), (iv), and (v) and the tetracycline hydrochloride used conforms to the requirements of § 446.81a(a)(1) except paragraphs (a)(1)(ii), (iv), and (v). Each other buffer and diluent used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* The immediate container shall be a tight container as defined by the U.S.P.; its closure shall be one through which a hypodermic needle cannot be introduced; and the container shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded. Each such container shall contain not less than 5 milligrams, and each may be packaged in combination with a container of the solvent, sterile distilled water U.S.P.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on the outside wrapper or container and the immediate container the statement "Expiration date ----------", the blank being filled in with the date that is 48 months, if it is chlortetracycline ophthalmic; or if it is tetracycline hydrochloride ophthalmic, the blank is filled in with the date that is 12 months after the month during which the batch was certified, except that the blank may be filled in with the date that is 24 months, 36 months, 48 months, or 60 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time, such drug as prepared by him complies with the standards prescribed by paragraph (a)(1) of this section.

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless previously submitted) the date on which the latest assay of the chlortetracycline or tetracycline hydrochloride used in making such batch was completed, the number of milligrams in each immediate container, the quantity of each buffer and diluent used in making the batch, the date on which the latest assay of the drug comprising such batch was completed, and a statement that each buffer and diluent used in making the batch conform to the requirements prescribed therefor, if any, by this section. If such batch or any part thereof is to be packaged with a solvent, such request shall also be accompanied by a statement that such solvent conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided in paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request results of the tests and assays listed after each of the following, made by him on an accurately representative sample of:

(*a*) The batch; potency, sterility, moisture, and pH of the solution prepared as directed in its labeling.

(*b*) The chlortetracycline or tetracycline hydrochloride used in making the batch; potency, toxicity, moisture, pH, crystallinity, and absorptivity, if it is tetracycline hydrochloride.

(iii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch:

(*1*) For all tests except sterility: One immediate container for each 5,000 immediate containers in the batch, but in no case less than five immediate containers.

Such samples shall be collected by taking single immediate containers at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(*2*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(*b*) The chlortetracycline or tetracycline hydrochloride used in making the batch: ten packages, each containing approximately equal portions of not less than 60 milligrams packaged in accordance with the requirements of § 446.10a(a)(2).

(c) In case of an initial request for certification, each buffer and diluent used in making the batch, one package of each containing approximately 5 grams.

(iv) No result referred to in paragraph (a)(4)(ii)(*b*) of this section, and no sample referred to in paragraph (a)(4)(iii)(*b*) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay—*(1) *Potency.* If it is tetracycline hydrochloride ophthalmic, proceed as directed in § 446.81a(b)(1); if it is chlortetracycline ophthalmic, proceed as directed in § 446.10a(b)(1). Its potency is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) of this chapter.

(3) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(4) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using a solution prepared as directed in the labeling of the drug.

§ 446.310b Chlortetracycline hydrochloride ointment; chlortetracycline calcium ointment; chlortetracycline calcium cream; tetracycline hydrochloride ointment (tetracycline hydrochloride in oil suspension); tetracycline ointment (tetracycline cream).

The requirements for certification and the tests and methods of assay for chlortetracycline hydrochloride ointment; chlortetracycline calcium ointment; chlortetracycline calcium cream; tetracycline hydrochloride ointment (tetracycline hydrochloride in oil suspension); tetracycline ointment (tetracycline cream) are described in § 446.510a.

§ 446.367 Oxytetracycline hydrochloride ophthalmic dosage forms.

§ 446.367a Ophthalmic oxytetracycline hydrochloride.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality and purity.* Ophthalmic oxytetracycline hydrochloride is a dry mixture of oxytetracycline hydrochloride, a mild astringent agent, and a buffer substance. It is sterile. Its moisture content is not more than 5.0 percent. The oxytetracyline hydrochloride is of such quantity that when dissolved as directed in the labeling the potency of such solution is 5.0 milligrams of oxytetracycline per milliliter. Such solution has a pH of not

less than 7.0 and not more than 8.5. The oxytetracycline hydrochloride used conforms to the standards prescribed by § 446.67a(a)(1) (i), (iii), (vi), (vii), (viii), and (ix). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* In addition to the labeling prescribed by § 432.5 of this chapter, each package shall bear on the outside wrapper or container a statement giving the method of dissolving the dry mixture and the statement "After reconstitution, the solution is stable for 48 hours under refrigeration." The expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline hydrochloride used in making the batch for potency, toxicity, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency, sterility, moisture, and pH.

(ii) Samples required:

(*a*) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 6 immediate containers.

(*2*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 446.65a(b)(1) (i) or (ii), except prepare the sample as follows: Reconstitute the sample as directed in the labeling. Dilute a representative aliquot in 0.1*N* hydrochloric acid to give a convenient stock solution. Dilute further in 0.1*M* potassium phosphate buffer, pH 4.5, to the prescribed reference concentration. The potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of oxytetracycline it is represented to contain.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) of this chapter.

(4) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using the solution obtained when prepared as directed in the labeling of the drug.

§ 446.367b Oxytetracycline hydrochloride ophthalmic oil suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride ophthalmic oil suspension is oxytetracycline hydrochloride in a suitable and harmless oil base. It may contain one or more suitable and harmless preservatives and stabilizing agents. Its potency is 10 milligrams of oxytetracycline per milliliter. Its moisture content is not more than 1.0 percent. The oxytetracycline hydrochloride used conforms to the standards prescribed by § 446.67a(a)(1) (i), (iii), (vi), (vii), (viii), and (ix). Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Requests for certification.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline hydrochloride used in making the batch for potency, toxicity, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 446.65a(b)(1) (i) or (ii), except prepare the sample as follows: Place a representative portion of the sample (usually 1.0 milliliter, accurately measured) in a glass blending jar containing 1.0 milliliter of polysorbate 80 and 199 milliliters of 0.1*N* hydrochloric acid. Using a high-speed blender, blend the mixture for approximately 3 minutes and make proper estimated dilutions to the prescribed reference concentration in 0.1*M* potassium phosphate buffer, pH 4.5. Its potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of oxytetracycline that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 446.367c Oxytetracycline hydrochloride-hydrocortisone acetate ophthalmic and otic suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride-hydrocortisone acetate ophthalmic and otic suspension is oxytetracycline hydrochloride and hydrocortisone acetate in a suitable and harmless oil base containing aluminum tristearate. Its potency is 5 milligrams of oxytetracycline per milliliter. It contains 15 milligrams of hydrocortisone acetate per milliliter. It is sterile. Its moisture content is not more than 1 percent. The oxytetracycline hydrochloride used conforms to the standards prescribed by § 446.67a(a)(1) (i), (iii), (vi), (vii), (viii), and (ix). Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Requests for certification.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) Oxytetracycline hydrochloride used in making the batch for potency, toxicity, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency, sterility, and moisture.

(ii) Samples required:

(*a*) Oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 5 immediate containers.

(*2*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 446.65a(b)(1) (i) or (ii), except prepare the sample as follows: Place a representative portion of the sample (usually 1.0 milliliter, accurately measured) in a glass blending jar containing 1.0 milliliter of polysorbate 80 and 199 milliliters of 0.1*N* hydrochloric acid. Using a high-speed blender, blend the mixture for approximately 3 minutes and make proper estimated dilutions to the prescribed reference concentration in 0.1*M* potassium phosphate buffer, pH 4.5. The potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of oxytetracycline that the suspension is represented to contain.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except use 0.25 milliliter of the sample in lieu of 1 milliliter.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 446.367d Oxytetracycline hydrochloride ophthalmic ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride ophthalmic ointment is oxytetracycline hydrochloride in a suitable and harmless ointment base. Its potency is 10 milligrams of oxytetracycline per gram. It is sterile. Its moisture content is not more than 1 percent. The oxytetracycline hydrochloride used conforms to the standards prescribed by § 446.67a(a)(1) (i), (iii), (vi), (vii), (viii), and (ix). Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of

§ 432.5 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline hydrochloride used in making the batch for potency, toxicity, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency, sterility, and moisture.

(ii) Samples required:

(*a*) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of five immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 446.65a (b) (1) (i) or (ii), except prepare the sample as follows: Place an accurately weighed representative portion of the sample in a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake ointment and ether until homogeneous. Shake with 25 milliliters of 0.1*N* hydrochloric acid. Remove the acid layer and repeat the extraction with each of three more 25-milliliter quantities of 0.1*N* hydrochloric acid. Combine the acid extractives, and make the proper estimated dilutions to the prescribed reference concentration in 0.1*M* potassium phosphate buffer, pH 4.5. The weighed sample may also be prepared by placing it in a glass blending jar containing 1.0 milliliter of polysorbate 80 and 199 milliliters of 0.1*N* hydrochloric acid. Using a high-speed blender, blend the mixture for approximately 3 minutes and make proper estimated dilutions to the prescribed reference concentration in 0.1*M* potassium phosphate buffer, pH 4.5. The potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of oxytetracycline that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e) (3) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 446.367e Oxytetracycline hydrochloride-polymyxin B sulfate eye and ear ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride-polymyxin B sulfate eye and ear ointment is oxytetracycline hydrochloride and polymyxin B sulfate in a suitable and harmless ointment base. Each gram of ointment contains 5 milligrams of oxytetracycline and 10,000 units of polymyxin B. Its moisture content is not more than 1 percent. The oxytetracycline hydrochloride used conforms to the standards prescribed by § 446.67a(a) (1) (i), (iii), (vi), (vii), (viii), and (ix). The polymyxin B sulfate used conforms to the standards prescribed by § 448.30a (a) (1) (i), (iv), (v), (vi), (vii), and (ix) of this chapter. Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline hydrochloride used in making the batch for potency, toxicity, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, toxicity, pH, moisture, residue on ignition, and identity.

(*c*) The batch for oxytetracycline content, polymyxin B content, sterility, and moisture.

(ii) Samples required:

(*a*) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) Polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The batch:

(*1*) For all tests except sterility: A minimum of six immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Oxytetracycline content.* Proceed as directed in § 446.567a(b) (1). Its oxytetracycline content is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of oxytetracycline that it is represented to contain.

(ii) *Polymyxin B content.* Proceed as directed in § 446.567b(b) (1) (ii). Its polymyxin B content is satisfactory if it contains not less than 90 percent and not more than 125 percent of the number of units of polymyxin B that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e) (3) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

Subpart E—Otic Dosage Forms

§ 446.467 Oxytetracycline hydrochloride otic dosage forms.

§ 446.467a Oxytetracycline hydrochloride-polymyxin B sulfate-benzocaine for otic solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride-polymyxin B sulfate-benzocaine for otic solution is a dry powder of oxytetracycline hydrochloride, polymyxin B sulfate, and benzocaine, packaged in combination with a suitable diluting solution which contains a preservative. When prepared as directed in the labeling, each milliliter contains 5.0 milligrams of oxytetracycline, 10,000 units of polymyxin B, and 50 milligrams of benzocaine. The moisture content of the powder is not more than 3.0 percent. The oxytetracycline hydrochloride used conforms to the standards prescribed by § 446.67a(a) (1) (i), (vi), (vii), (viii), and (ix). The polymyxin B sulfate used conforms to the standards prescribed by § 448.30a(a) (1) (i), (v), (vi), (vii), and (ix) of this chapter. Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline hydrochloride used in making the batch for potency, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, moisture, pH, residue on ignition, and identity.

(*c*) The batch for oxytetracycline content, polymyxin content, and moisture content of the powder.

(ii) Samples required:

(*a*) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The batch: A minimum of seven immediate containers.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Oxytetracycline content.* Proceed as directed in § 446.65a(b) (1) (i) or (ii), except prepare the sample for assay as follows: Reconstitute as directed in the labeling. Dilute 5.0 milliliters of this reconstituted sample to a volume of 100 milliliters with 0.1*N* hydrochloric acid. Make proper estimated dilutions to the prescribed reference concentration with 1.0*M* potassium phosphate buffer, pH 4.5. The content of oxytetracycline is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of oxytetracycline that it is represented to contain.

(ii) *Polymyxin content.* Proceed as directed in § 446.567c(b) (1) (ii) (*a*) or (*b*), except use the entire contents of the powder from each vial tested. Reconstitute a separate vial as directed in the labeling, measure the total volume that can be withdrawn, and, from the polymyxin content of the powder, calculate

the number of units per milliliter. The content of polymyxin is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of units of polymyxin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 440.74a(b)(5) of this chapter.

§ 446.467b Oxytetracycline hydrochloride-polymyxin B sulfate eye and ear ointment.

The requirements for certification and the tests and methods of assay for oxytetracycline hydrochloride-polymyxin B sulfate eye and ear ointment are described in § 446.367e.

§ 446.467c Oxytetracycline hydrochloride-hydrocortisone acetate ophthalmic and otic suspension.

The requirements for certification and the tests and methods of assay for oxytetracycline hydrochloride-hydrocortisone acetate ophthalmic and otic suspension are described in § 446.367c.

§ 446.481 Tetracycline hydrochloride otic (tetracycline hydrochloride for ear solution).

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Tetracycline otic is a packaged combination of one immediate container of crystalline tetracycline hydrochloride and one immediate container of a suitable and harmless solution. The tetracycline hydrochloride is of such quantity that, when dissolved as directed, the potency of such solution is not less than 5 milligrams per milliliter after it has been kept for 7 days at a temperature of 15° C. (59° F.). The tetracycline hydrochloride used conforms to the requirements of § 446.81a(a)(1), except § 446.81a(a)(1)(ii), (iii), (iv), and (v). Each substance used in the preparation of the solution contained in the packaged combination, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* Each immediate container shall be a tight container as defined by the U. S. P. and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106 (b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on the label or labeling, as hereinafter indicated, the following:

(i) On the outside wrapper or container and on the immediate container of the tetracycline hydrochloride, the statement "Expiration date _____", the blank being filled in with the date that is 36 months after the month during which the batch was certified.

(ii) On the outside wrapper or container and on the immediate container of the solution in the packaged combination, a statement giving the method of dissolving the tetracycline hydrochloride in the solution and the conditions under which the solution should be stored, including reference to its instability when stored under other conditions, and the statement, "The solution may be kept in a refrigerator for 1 week without significant loss of potency".

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the number of milligrams in each immediate container thereof, the date on which the latest assay of the batch was completed, the batch mark, and (unless it was previously submitted) the date on which the latest assay of the tetracycline hydrochloride used in making such batch was completed, the quantity of each ingredient used in making the solution included in the packaged combination, and a statement that such solution conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided in paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request results of the tests and assays listed after each of the following, made by him on an accurately representative sample of:

(*a*) The batch; potency and moisture.

(*b*) The solution after the tetracycline hydrochloride has been dissolved therein: potency.

(*c*) The tetracycline hydrochloride used in making the batch: potency, moisture, pH, crystallinity, and absorptivity.

(iii) Except as otherwise provided in paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch; one package for each 5,000 packages in the batch, but in no case less than 5 packages, collected by taking a single package at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(*b*) The tetracycline hydrochloride used in making the batch: 10 packages, each containing approximately equal portions of not less than 60 milligrams, packaged in accordance with the requirements of § 446.10a(a)(2).

(*c*) In case of an initial request for certification, or when any change is made in composition of such solution; 5 packages of the solution included in the combination.

(iv) No result referred to in paragraph (a)(4)(ii)(*c*) of this section, and no sample referred to in paragraph (a)(4)(iii)(*b*) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 446.81a(b)(1).

(2) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) of this chapter.

Subpart F—Dermatologic Dosage Forms

§ 446.510 Chlortetracycline dermatologic dosage forms.

§ 446.510a Chlortetracycline hydrochloride ointment; chlortetracycline calcium ointment; chlortetracycline calcium cream; tetracycline hydrochloride ointment (tetracycline hydrochloride in oil suspension); tetracycline ointment (tetracycline cream).

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Chlortetracycline hydrochloride ointment, tetracycline hydrochloride ointment, and tetracycline ointment are crystalline chlortetracycline hydrochloride, chlortetracycline calcium, tetracycline hydrochloride, or tetracycline, in a suitable and harmless ointment base. It may contain a suitable local anesthetic, cortisone, hydrocortisone, or a suitable ester of cortisone or hydrocortisone, and one or more suitable and harmless preservatives and stabilizing agents. If it is intended for ophthalmic use, it contains chlortetracycline hydrochloride or tetracycline hydrochloride and it is sterile. Its moisture content is not more than 1 percent if it is chlortetracycline hydrochloride or tetracycline hydrochloride ointment. Its potency is not less than 1 milligram per gram. The chlortetracycline hydrochloride used in making the chlortetracycline hydrochloride ointment and in preparing the chlortetracycline calcium used in making the chlortetracycline calcium ointment conforms to the requirements of § 446.10a(a)(1) except § 446.10a(a)(1)(i), (ii), (iii), (iv), and (v) but its potency is not less than 750 micrograms per milligram. The chlortetracycline hydrochloride used in making the chlortetracycline hydrochloride ophthalmic ointment conforms to the requirements of § 446.10a(a)(1) except § 446.10a(a)(1)(iv) and (v). The tetracycline hydrochloride used conforms to the requirements of § 446.81a(a)(1) except § 446.81a(a)(1)(ii), (iii), (iv), and (v). The tetracycline hydrochloride used in making the tetracycline hydrochloride ophthalmic ointment conforms to the requirements of § 446.81a(a)(1) except § 446.81a(a)(1)(iv) and (v). The tetracycline used conforms to the requirements of § 446.80(a)(1). Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* The ointment shall be packaged in collapsible tubes which are well-closed containers as defined by the U. S. P., or in containers of glass or plastic that are tight containers as defined by the U. S. P. Unless it is labeled solely for hospital use, each such container shall contain not more than 2 ounces, except if it is intended for ophthalmic use each such container shall contain not more than ⅛-ounce, if it is a collapsible tube, or more than ½-ounce if it is a tight container. Each such container shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor

changes so caused that are normal and unavoidable in good packaging, storage, and distribution practices shall be disregarded.

(3) *Labeling*—(i) *It is packaged for ophthalmic use or it contains cortisone, hydrocortisone, or an ester of cortisone or hydrocortisone.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its label or labeling, as hereinafter indicated, the following:

(a) The statement "Expiration date _____", the blank being filled in with the date that is 48 months if it is chlortetracycline hydrochloride ointment or tetracycline hydrochloride ointment, or 24 months if it is chlortetracycline calcium ointment, after the month during which the batch was certified, except that the blank may be filled in with the date that is 36 months, 48 months, or 60 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time the drug as prepared by him complies with the standards prescribed by paragraph (a) (1) of this section.

(b) If it contains one or more of the active ingredients specified in paragraph (a)(1) of this section, after the name "chlortetracycline ointment", "chlortetracycline calcium ointment", "chlortetracycline calcium cream", "tetracycline hydrochloride ointment", or "tetracycline ointment", wherever it appears, the words "with _____", in juxtaposition with such name, the blank being filled in with the established name of each such ingredient used.

(ii) *It is not packaged for ophthalmic use, and it does not contain cortisone, hydrocortisone, or an ester of cortisone or hydrocortisone.* Its label and labeling shall comply with all the requirements prescribed by paragraph (a)(3)(i) of this section, except that in lieu of the statement "Caution: Federal law prohibits dispensing without prescription", each package shall contain a circular or other labeling within or attached to the package bearing adequate directions and warnings for the prophylactic use of such ointment.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The chlortetracycline hydrochloride or tetracycline hydrochloride or tetracycline used in making the batch for potency, moisture, pH, and crystallinity, and for absorptivity if it is tetracycline hydrochloride or tetracycline, and for toxicity if the ointment is intended for ophthalmic use.

(b) The batch for potency and moisture and for sterility if it is intended for ophthalmic use.

(ii) Samples required:

(a) The chlortetracycline or tetracycline hydrochloride or tetracycline used in making the batch: 10 packages, each containing approximately 60 milligrams, packaged in accordance with the requirements of § 446.10a(a)(2).

(b) The batch:

(1) For all tests except sterility: A minimum of 5 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* If it is tetracycline hydrochloride ointment or tetracycline ointment, proceed as directed in § 446.81a(b)(1), and if it is chlortetracyline ointment proceed as directed in § 446.10a(b)(1), except § 446.10a(b)(1)(x), and in lieu of the directions in § 446.10a(b)(1)(iv) and (viii) (c) prepare the sample as follows: Place a representative portion of the sample (usually approximately 1.0 gram, accurately weighed) in a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake ointment and ether until homogeneous. Shake with a 25-milliliter portion of 0.01*N* HCl. Remove the acid layer and repeat the extraction with three 25-milliliter quantities of 0.01*N* HCl. Combine the extracts and make the proper estimated dilutions in 0.1*M* monopotassium phosphate buffer, pH 4.5. The sample may also be prepared by placing a representative portion of the sample (usually 1.0 gram, accurately weighed) in a glass blending jar containing 200 milliliters of 0.01*N* HCl. Using a high-speed blender, blend the mixture for approximately 2 minutes and make proper estimated dilutions in 0.1*M* monopotassium phosphate buffer, pH 4.5. The potency is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Sterility.* If the ointment is intended for ophthalmic use, proceed as directed in § 436.20 of this chapter, using the method described in paragraph (c)(3) of that section.

§ 446.510b Chlortetracycline powder (chlortetracycline hydrochloride powder); tetracycline hydrochloride powder; tetracycline powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chlortetracycline powder, tetracycline hydrochloride powder, and tetracycline powder are crystalline chlortetracycline hydrochloride, tetracycline hydrochloride, or tetracycline, with or without glucosamine hydrochloride and with or without suitable and harmless buffer substances, preservatives, diluents, colorings, and flavorings. The content of chlortetracycline or tetracycline hydrochloride or tetracycline is not less than 15 milligrams per gram of powder. The moisture content of chlortetracycline powder or tetracycline hydrochloride powder is not more than 2 percent. The moisture content of tetracycline powder is not more than 7.5 percent. The chlortetracycline used conforms to the requirements of § 446.10a(a)(1), except § 446.10a(a)(1)(ii), (iv) and (v). The tetracycline hydrochloride used conforms to the requirements of § 446.81a(a)(1), except paragraphs (a)(1)(ii), (iv), and (v) of that section. The tetracycline used conforms to the requirements of § 446.80(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* In all cases the immediate container shall be a tight container as defined by the U.S.P. The composition of the immediate container shall be such as will not cause any change in the strength, quality, or purity of the contents beyond any limits therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on the outside wrapper or container and the immediate container, as hereinafter indicated, the following:

(i) The statement "Expiration date _____", the blank being filled in with the date that is 60 months after the month during which the batch was certified if it is chlortetracycline hydrochloride powder; or if it is tetracycline hydrochloride powder or tetracycline powder the blank is filled in with the date that is 24 months after the month during which the batch was certified, except that the blank may be filled in with the date that is 36 months, 48 months, or 60 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time such drug as prepared by him complies with the standards prescribed by paragraph (a)(1) of this section.

(ii) If it contains, in addition to chlortetracycline hydrochloride, tetracycline hydrochloride, or tetracycline, one or more of the other active ingredients specified in paragraph (a)(1) of this section, after the name "chlortetracycline powder", "tetracycline hydrochloride powder", or "tetracycline powder", wherever such name appears, the words "with _____ (the blank being filled in with the established name of each other such ingredient)", in juxtaposition with such name.

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless it was previously submitted) the date on which the latest assay of the chlortetracycline, tetracycline hydrochloride or tetracycline used in making such batch was completed, the number of milligrams in each immediate container, the quantity of each ingredient used in making the batch, the date on which the latest assay of the drug compris-

ing such batch was completed, and a statement that each ingredient used in making the batch conforms to the requirements prescribed therefor, if any, by this section.

(ii) Except as otherwise provided in paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request results of the tests and assays listed after each of the following, made by him on an accurately representative sample of:

(*a*) The batch; potency and moisture.

(*b*) The chlortetracycline, tetracycline hydrochloride, or tetracycline used in making the batch; potency, toxicity, moisture, pH, crystallinity, and absorptivity if it is tetracycline hydrochloride or tetracycline.

(iii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch; one immediate container for each 5,000 containers in the batch, but in no case less than 5 immediate containers, unless each such container is packaged to contain more than 1.0 gram, in which case the sample shall consist of 1.0 gram for each 5,000 immediate containers in the batch, but in no case less than five 1.0-gram portions, except if it is intended for use as an ingredient in the drinking water of animals, each portion in the sample submitted shall consist of 1 ounce in lieu of 1.0 gram. Such sample shall be collected by taking single immediate containers, 1.0-gram portions, or 1-ounce portions at such intervals throughout the entire time the containers are being filled that the quantities filled during the intervals are approximately equal.

(*b*) The chlortetracycline, tetracycline hydrochloride, or tetracycline used in making the batch; 10 packages, each containing approximately equal portions of not less than 60 milligrams, packaged in accordance with the requirements of § 446.10a(a)(2).

(c) In case of an initial request for certification, each other ingredient used in making the batch; one package of each containing approximately 5 grams.

(iv) No result referred to in paragraph (a)(4)(ii)(*b*) of this section, and no sample referred to in paragraph (a)(4)(iii)(*b*) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay*—(1) *Potency.* Using a 3.0-gram sample or the entire contents of the immediate container for each determination, proceed as directed in § 446.81a(b)(1), if it is chlortetracycline powder. If it is tetracycline powder, blend a 3.0-gram sample as directed in § 436.513(a), or reconstitute in the immediate container as directed in the labeling of the drug. Transfer an appropriate aliquot of 1.0 milliliter to 5.0 milliliters to a 100-milliliter volumetric flask and make to mark with 0.1*N* HCl. Withdraw an aliquot from the volumetric flask and dilute to 0.24 microgram per milliliter, using *M*/10 phosphate buffer at pH 4.5 and proceed as directed in § 446.81a(b)(1). The average potency of the powder is satisfactory if it contains not less than 85 percent of the number of milligrams of chlortetracycline or tetracycline hydrochloride or tetracycline per gram or per immediate container that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) of this chapter.

§ 446.567 Oxytetracycline hydrochloride dermatologic dosage forms.

§ 446.567a Oxytetracycline hydrochloride-hydrocortisone topical ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride-hydrocortisone topical ointment is oxytetracycline hydrochloride and hydrocortisone in a suitable and harmless ointment base. Its potency is 30 milligrams of oxytetracycline per gram. It contains 10 milligrams of hydrocortisone per gram. Its moisture content is not more than 1 percent. The oxytetracycline hydrochloride used conforms to the standards prescribed by § 446.67a(a)(1)(i), (vi), (vii), (viii), and (ix). Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline hydrochloride used in making the batch for potency, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(c) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 446.65a(b)(1)(i) or (ii), except prepare the sample as follows: Place an accurately weighed representative portion of the sample in a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake ointment and ether until homogeneous. Shake with 25 milliliters of 0.1*N* hydrochloric acid. Remove the acid layer, and repeat the extraction with each of three more 25-milliliter quantities of 0.1*N* hydrochloric acid. Combine the extractives, and make the proper estimated dilutions to the prescribed reference concentration in 0.1*M* potassium phosphate buffer, pH 4.5. The weighed sample may also be prepared by placing it in a glass blending jar containing 1.0 milliliter of polysorbate 80 and 199 milliliters of 0.1*N* hydrochloric acid. Using a high-speed blender, blend the mixture for approximately 3 minutes and make proper estimated dilutions to the prescribed reference concentration in 0.1*M* potassium phosphate buffer, pH 4.5. The potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of oxytetracycline that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 446.567b Oxytetracycline hydrochloride-polymyxin B sulfate topical ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride-polymyxin B sulfate topical ointment is oxytetracycline hydrochloride and polymyxin B sulfate in a suitable and harmless ointment base. Each gram of topical ointment contains 30 milligrams of oxytetracycline and 10,000 units of polymyxin B. Its moisture content is not more than 1 percent. The oxytetracycline hydrochloride used conforms to the standards prescribed by § 446.67a(a)(1)(i), (vi), (vii), (viii), and (ix). The polymyxin B sulfate conforms to the standards prescribed by § 448.30a(a)(1)(i), (v), (vi), (vii), and (ix) of this chapter. Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* Each package shall bear, on its label or labeling, as hereinafter indicated, the following:

(i) On the label of the immediate container and on the outside wrapper or container, if any:

(*a*) The batch mark.

(*b*) The name and quantity of each active ingredient contained in the drug.

(*c*) An expiration date which is 12 months after the month during which the batch was certified.

(ii) On the label of the immediate container or other labeling attached to or within the package: Adequate directions under which the layman can use the drug safely and efficaciously.

(3) *Request for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline hydrochloride used in making the batch for potency, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, moisture, pH, residue on ignition, and identity.

(*c*) The batch for oxytetracycline content, polymyxin B content, and moisture.

(ii) Samples required:

(*a*) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(c) The batch: A minimum of 6 immediate containers.

(d) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Oxytetracycline content.* Proceed as directed in § 446.567a(b)(1). The oxytetracycline content is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of oxytetracycline that it is represented to contain.

(ii) *Polymyxin B content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay by either of the following methods:

(a) Weigh accurately about 1 gram of the ointment and place in a 50-milliliter centrifuge tube. Add 15 milliliters of ethyl ether. Stir until contents are homogeneous. Add 25 milliliters of ethyl ether, stir, and centrifuge for 10 minutes at 3000 revolutions per minute. Decant the supernatant ether. Add 20 milliliters of acetone and stir until contents are homogenous. Add 25 milliliters of acetone and centrifuge for 10 minutes at 3000 revolutions per minute. Decant the supernatant acetone and repeat acetone extraction twice more, discarding the acetone supernatant each time. Wash the residue into a 100-milliliter volumetric flask with 10 percent potassium phosphate buffer, pH 6.0, and dilute with this same buffer to a final estimated concentration of 10 units of polymyxin B per milliliter.

(b) Weigh accurately about 1 gram of the ointment and place in a filter-funnel using a solvent-resistant membrane filter of 1.5 micron porosity. Wash with five 20-milliliter portions of warm benzene. Wash residue with five 20-milliliter portions of acetone or until yellow color has disappeared. Remove the filter and soak in about 50 milliliters of 10 percent potassium phosphate buffer, pH 6.0, for one-half hour to one hour. Transfer the solution quantitatively to a 100-milliliter volumetric flask and bring to mark with 10 percent potassium phosphate buffer, pH 6.0. Make appropriate dilutions with 10 percent potassium phosphate buffer, pH 6.0, to the prescribed reference concentration.

Its polymyxin B content is satisfactory if it contains not less than 90 percent and not more than 125 percent of the number of units of polymyxin B that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 446.567c Oxytetracycline hydrochloride-polymyxin B sulfate topical powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride-polymyxin B sulfate topical powder is oxytetracycline hydrochloride and polymyxin B sulfate with a suitable filler. Each gram contains 30 milligrams of oxytetracycline and 10,000 units of polymyxin B. The moisture content is not more than 2.0 percent. The oxytetracycline hydrochloride used conforms to the standards prescribed by § 446.67a(a)(1)(i), (vi), (vii), (viii), and (ix). The polymyxin B sulfate used conforms to the standards prescribed by § 448.30a(a)(1)(i), (v), (vi), (vii), and (ix) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* Each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the label of the immediate container and on the outside wrapper or container, if any:

(a) The batch mark.

(b) The name and quantity of each active ingredient contained in the drug.

(c) An expiration date that is 12 months after the month during which the batch was certified, unless the use of a longer expiration period has been approved in accordance with the provisions of § 432.5(a)(3) of this chapter.

(ii) On the label of the immediate container or other labeling attached to or within the package, adequate directions for lay use of the drug.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The oxytetracycline hydrochloride used in making the batch for potency, moisture, pH, absorptivity, crystallinity, and identity.

(b) The polymyxin B sulfate used in making the batch for potency, moisture, pH, residue on ignition, and identity.

(c) The batch for oxytetracycline content, polymyxin content, and moisture.

(ii) Samples required:

(a) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(c) The batch: A minimum of six immediate containers.

(d) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Oxytetracycline content.* Proceed as directed in § 446.65a(b)(1)(i) or (ii), except prepare the sample for assay as follows: Place an accurately weighed representative portion in a 1-liter volumetric flask, dissolve, and dilute to volume with 0.1*N* hydrochloric acid. Make the proper estimated dilutions of an aliquot of the stock solution to the prescribed reference concentration in 0.1*M* potassium phosphate buffer, pH 4.5. The content of oxytetracycline is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of oxytetracycline that it is represented to contain.

(ii) *Polymyxin content.* Proceed as directed in § 448.30a(b)(1) of this chapter, except prepare the sample for assay by either of the following methods:

(a) Accurately weigh approximately 1 gram of the powder and place in a 50-milliliter centrifuge tube. Add 15 milliliters of acetone and 1 drop of concentrated hydrochloric acid and stir well. Add 20 milliliters of acetone and centrifuge for 10 minutes at 3,000 revolutions per minute. Decant the supernatant liquid and repeat the acetone-acid extraction once more. Dissolve and dilute the residue with sufficient 10 percent potassium phosphate buffer, pH 6.0, to give a stock solution of convenient concentration. Remove an aliquot and make proper estimated dilutions to the prescribed reference concentration with 10 percent potassium phosphate buffer, pH 6.0.

(b) Place the accurately weighed sample in a filter funnel with a solvent-resistant membrane filter of 1.5μ porosity. Wash the powder with five 20-milliliter portions of acetone or until yellow color has disappeared. Remove the filter and soak in an adequate volume of 10 percent potassium phosphate buffer, pH 6.0. Quantitatively transfer to a 100-milliliter volumetric flask and adjust to volume with 10 percent potassium phosphate buffer, pH 6.0. Make proper estimated dilutions with 10 percent potassium phosphate buffer, pH 6.0, to the prescribed reference concentration.

The content of polymyxin is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of units of polymyxin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 440.20a(b)(5)(i) of this chapter.

§ 446.567d Oxytetracycline hydrochloride–hydrocortisone aerosol topical.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride–hydrocortisone aerosol topical is oxytetracycline hyrochloride and hydrocortisone in a suitable ointment base, packaged with one or more suitable inert gases. Each spray pack contains 300 milligrams of oxytetracycline and 100 milligrams of hydrocortisone. The moisture content is not more than 1.0 percent. The oxytetracycline hydrochloride used conforms to the standards prescribed by § 446.67a(a)(1)(i), (vi), (vii), (viii), and (ix). Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The oxytetracycline hydrochloride used in making the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(b) The batch for potency and moisture.

(ii) Samples required:

(a) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch: A minimum of five immediate containers.

(c) In case of an initial request for certification each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 446.65a (b)(1) (i) or (ii), except prepare the sample for assay as follows: Remove the cap and plastic spray tip from the aerosol can. Attach a 10-inch length of suitable plastic tubing over the nozzle of the aerosol can. Shake the can gently two or three times, place the free end of the tubing into a 400-milliliter beaker, hold the can in an upright position and depress the nozzle. Empty the entire contents into the beaker. Allow to stand at room temperature for ½ to 1 hour to evaporate residual propellant. Rinse the tubing with a minimum amount of 0.1*N* hydrochloric acid and add it to the contents in the beaker. Transfer the contents of the beaker with three 10-milliliter portions of 0.1*N* hydrochloric acid to a 1-liter volumetric flask. Bring to volume with 0.1*N* hydrochloric acid and mix well. Remove an appropriate aliquot of the remaining filtrate and, using 0.1*M* potassium phosphate buffer, pH 4.5, make proper estimated dilutions to the prescribed reference concentration. Its content of oxytetracycline is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of oxytetracycline that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141b.117(c) of this chapter.

§ 446.567e Oxytetracycline hydrochloride-polymyxin B sulfate-hydrocortisone aerosol topical.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride-polymyxin B sulfate-hydrocortisone aerosol topical is oxytetracycline hydrochloride, polymyxin B sulfate, and hydrocortisone in a suitable ointment base, packaged with one or more suitable inert gases. Each spray pack contains 300 milligrams of oxytetracyline, 100,000 units of polymyxin B, and 100 milligrams of hydrocortisone. The moisture content is not more than 1.0 percent. The oxytetracycline hydrochloride used conforms to the standards prescribed by § 446.67a (a)(1) (i), (vi), (vii), (viii), and (ix). The polymyxin B sulfate used conforms to the standards prescribed by § 448.30a (a)(1) (i), (v), (vi), (vii), and (ix) of this chapter. Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The oxytetracycline hydrochlorids used in making the batch for potency, moisture, pH, absorptivity, crystallinity, and identity.

(b) The polymyxin B sulfate used in making the batch for potency, moisture, pH, residue on ignition, and identity.

(c) The batch for oxytetracycline content, polymyxin content, and moisture.

(ii) Samples required:

(a) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(c) The batch: A minimum of six immediate containers.

(d) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Oxytetracycline content.* Proceed as directed in § 446.567d(b)(1) of this chapter. The content of oxytetracycline is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of oxytetracycline that it is represented to contain.

(ii) *Polymyxin content.* Proceed as directed in § 448.30a(b)(1) of this chapter, except prepare the sample for assay as follows: Remove the cap and plastic spray tip from the aerosol can. Attach a 10-inch length of suitable plastic tubing over the nozzle and shake the can gently. Place the free end of the tubing into a filter funnel having a solvent-resistant membrane of 1.5μ porosity, hold the can upright, and depress the nozzle until the contents are completely emptied into the funnel. Evaporate any residual propellant by allowing it to stand at room temperature for ½ to 1 hour. Rinse the plastic tubing with small portions of acetone and pour the washings into the funnel. Wash the sample with five 20-milliliter portions of acetone or until yellow color has disappeared. Remove the filter and soak in an adequate volume with 10 percent potassium phosphate buffer, pH 6.0. Quantitatively transfer to a 100-milliliter volumetric flask and adjust to volume with 10 percent potassium phosphate buffer, pH 6.0. Make proper estimated dilutions with 10 percent potassium phosphate buffer, pH 6.0, to the prescribed reference concentration. The content of polymyxin is satisfactory if it contains not less than 90 percent and not more than 135 percent of the number of units of polymyxin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141b.117(c) of this chapter.

§ 446.581 Tetracycline hydrochloride dermatologic dosage forms.

§ 446.581a Tetracycline hydrochloride-neomycin spray ointment topical.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline hydrochloride-neomycin spray ointment topical is tetracycline hydrochloride and neomycin in a suitable and harmless ointment base and one or more suitable and harmless inert gases. Each gram of ointment contains not less than 15 milligrams of tetracycline hydrochloride and not less than 15 milligrams of neomycin. It is sterile. Its moisture content is not more than 3.0 percent. The tetracycline hydrochloride used conforms to the requirements of § 446.81a(a)(1), except § 446.81a(a)(1) (i), (ii), (iii), (iv), and (v), but its potency is not less than 720 μg. per milligram. The neomycin used conforms to the standards prescribed by § 444.42a(a)(1) (i), (v), and (vi) of this chapter. Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* In all cases the immediate container shall be a tight container as defined by the U. S. P. and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused that are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded. Each such container shall contain not less than 30 grams of ointment and shall contain one or more suitable and harmless inert gases.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on the outside wrapper or container and the immediate container the statement "Expiration date _____", the blank being filled in with the date that is 24 months after the month during which the batch was certified.

(4) *Request for cerification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless they were previously submitted) the dates on which the latest assays of the tetracycline hydrochloride and neomycin used in making such batch were completed, the quantity of each ingredient used in making such batch, the date on which the latest assay of the drug comprising such batch was completed, and that each ingredient used in making the batch conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request results of the tests and assays listed after each of the following, made by him on an accurately representative sample of:

(a) The batch: The number of milligrams of tetracycline hydrochloride and the number of milligrams of neomycin per gram; sterility; moisture.

(b) The tetracycline hydrochloride and neomycin used in making the batch: Potency, moisture, pH, crystallinity.

(iii) Except as otherwise provided in paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch:

(*1*) For all tests except sterility: One immediate container for each 5,000 immediate containers in the batch, but in no case less than six immediate containers.

(2) For sterility testing: 20 packages. Such samples shall be collected by taking single immediate containers at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(*b*) The tetracycline hydrochloride used in making the batch: 10 packages, each containing approximately equal portions of not less than 60 milligrams, packaged in accordance with the requirements of § 446.81a(a)(2).

(*c*) The neomycin used in making the batch: Five packages, each containing approximately equal portions of not less than 0.5 gram.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each component of the ointment base, each containing approximately 200 grams.

(iv) The results referred to in paragraph (a)(4)(ii)(*b*) of this section and the samples referred to in paragraph (a)(4)(iii) (*b*) and (*c*) of this section are not required if such results or samples have been previously submitted.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Tetracycline hydrochloride content.* Spray the entire contents of the well-shaken sample into a large tared beaker. Place in a vacuum desiccator, evacuate, and allow to stand at room temperature for 16 to 18 hours. After the gas has volatilized, accurately weigh the beaker and contents and determine the weight of the ointment ejected when used as directed in its labeling. Place an accurately weighed sample of approximately 1 gram in an extraction funnel prepared by fusing a ground-glass joint to the top of a medium-porosity sintered-glass filter funnel (30-millimeter diameter). Wash with five 10-milliliter portions of warm iso-octane and draw off the ointment base under vacuum. Discard the iso-octane washings. Wash the residue in the funnel four times with 10-milliliter portions of 0.3 percent piperidine in acetone solution. Withdraw each washing under vacuum. Combine the four washings in a 100-milliliter volumetric flask and make to mark with acetone. Remove an aliquot of this solution and proceed as directed in § 446.81a(b)(1). The sample may also be assayed by using a 1-gram sample weighed as described above and proceeding as directed in § 446.510a(b)(1). The tetracycline hydrochloride content is satisfactory if it is not less than 85 percent of that which it is represented to contain.

(ii) *Neomycin content.* The residue remaining in the funnel after the extraction described in paragraph (b)(1)(i) of this section contains the neomycin. Wash this residue five times, using 10-milliliter aliquots of 0.1*M* potassium phosphate buffer, pH 8.0, drawing each washing off under vacuum. Combine the washings in a 100-milliliter volumetric flash and make to mark with 0.1*M* potassium phosphate buffer, pH 8.0. Using an aliquot of this aqueous solution, proceed as directed in § 436.517(b) of this chapter. The sample may also be assayed by using a 1-gram sample weighed as described in paragraph (b)(1)(i) of this section and proceeding as directed in § 436.516(a)(2), except first prepare the sample by blending in an appropriate volume of 0.1*M* potassium phosphate buffer, pH 8.0, using a high-speed blender. The content of neomycin is satisfactory if it is not less than 85 percent of that which it is represented to contain.

(2) *Sterility.* Use 500 milligrams of ointment from each immediate container and proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(2) of that section.

(3) *Moisture.* Proceed as directed in § 141b.117(c) of this chapter, except dissolve the sample in 10 milliliters of dry chloroform.

§ 446.581b Tetracycline hydrochloride-neomycin in oil suspension.

(a) *Requirements for certification*—(1) Tetracycline hydrochloride-neomycin in oil suspension conforms to all requirements and is subject to all procedures prescribed by § 446.510a(a) for tetracycline hydrochloride ointment, except that:

(i) It contains not less than 10 milligrams of neomycin per milliliter. The neomycin used conforms to the standards prescribed by § 444.42a(a)(1)(i),(v), and (vi) of this chapter.

(ii) Its labeling shall also show the quantity of neomycin contained in each milliliter.

(2) In addition to complying with the requirements of § 446.510a(a)(4), a person who requests certification of a batch shall submit with his request a statement showing the batch mark and, unless it was previously submitted, the results and the date of the latest tests and assays of the neomycin used in making the batch for potency, moisture, and pH. He shall also submit in connection with his request a sample consisting of not less than 6 packages of such drug and, unless it was previously submitted, a sample consisting of 5 packages containing approximately equal portions of not less than 0.5 gram of the neomycin used in making such batch.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Content of tetracycline hydrochloride.* Proceed as directed in § 141c.237(a)(2) of this chapter or § 446.510a(b)(1). Its content of tetracycline hydrochloride is satisfactory if it is not less than 85 percent of that which it is represented to contain.

(ii) *Content of neomycin.* Proceed as directed in § 141c.237(a)(3) of this chapter or as directed in § 436.516(a)(2) of this chapter, except first prepare the sample by blending a 1-milliliter aliquot in an appropriate volume of 0.1*M* potassium phosphate buffer, pH 8.0, using a high-speed blender. Its content of neomycin is satisfactory if it is not less than 85 percent of that which it is represented to contain.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

Subpart G—Vaginal Dosage Forms

§ 446.610 Chlortetracycline suppositories (chlortetracycline hydrochloride suppositories); tetracycline hydrochloride suppositories.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chlortetracycline suppositories and tetracycline hydrochloride suppositories are suppositories composed of crystalline chlortetracycline or tetracycline hydrochloride in a suitable and harmless base, with or without one or more suitable and harmless preservatives. The potency of each suppository is not less than 100 milligrams, except if it is intended for rectal use its potency is not less than 50 milligrams. Its moisture content is not more than 1 percent. The chlortetracycline used conforms to the requirements of § 446.10a(a)(1), except § 446.10a(a)(1)(i), (ii), (iii), (iv), and (v), but its potency is not less than 750 micrograms per milligram. The tetracycline hydrochloride used conforms to the requirements of § 446.81a(a)(1), except § 446.81a(a)(1)(ii), (iii), (iv), and (v). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* In all cases the immediate container shall be a tight container as defined by the U. S. P., and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its outside wrapper or container and the immediate container the statement "Expiration date ----------", the blank being filled in with the date that is 24 months after the month during which the batch was certified, except that the blank may be filled in with the date that is 36 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time, such drug as prepared by him complies with the standards prescribed by paragraph (a)(1) of this section.

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certifica-

tion of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless it was previously submitted) the date on which the latest assay of the chlortetracycline or tetracycline hydrochloride used in making such batch was completed, the number of milligrams of chlortetracycline or tetracycline hydrochloride in each suppository, the date on which the latest assay of the drug comprising such batch was completed, the quantity of each ingredient used in making the batch, and a statement that each such ingredient conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request results of tests and assays listed after each of the following, made by him on an accurately representative sample of:

(*a*) The batch; average potency per suppository and moisture.

(*b*) The chlortetracycline or tetracycline hydrochloride used in making the batch; potency, moisture, pH, crystallinity, and absorptivity if it is tetracycline hydrochloride.

(iii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch; one suppository for each 5,000 suppositories in the batch, but in no case less than 30 suppositories, collected by taking single suppositories at such intervals throughout the entire time the suppositories are being made that the quantities made during the intervals are approximately equal.

(*b*) The chlortetracycline or tetracycline hydrochloride used in making the batch; 10 packages, each containing approximately equal portions of not less than 60 milligrams, packaged in accordance with § 446.10a(a)(2).

(*c*) In case of an initial request for certification, each other ingredient used in making the batch; one package of each containing approximately 5 grams.

(iv) No result referred to in paragraph (a)(4)(ii)(b) of this section, and no sample referred to in paragraph (a)(4)(iii)(*b*) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay*—(1) *Potency.* If they are tetracycline hydrochloride suppositories, proceed as directed in § 446.81a(b)(1); and if they are chlortetracycline suppositories, proceed as directed in § 446.10a(b)(1), except paragraph (b)(1)(x) of that section, and in lieu of the directions in paragraph (b)(1)(iv) and (viii)(*c*) of § 446.10a, prepare the sample as follows: Place three suppositories into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the suppositories and ether until homogeneous. Shake with a 50-milliliter portion of 0.01*N* HCl. Remove the aqueous layer and repeat the extraction with three 50-milliliter quantities of 0.01 *N* HCl. Combine the extractives and centrifuge an aliquot at 2,000 r.p.m. for 5 minutes. Remove an aliquot of the supernatant and make the proper estimated dilutions in the buffer solution. The average potency of the suppository is satisfactory if it contains not less than 85 percent of the number of milligrams of chlortetracycline or tetracycline hydrochloride that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 446.667 Oxytetracycline hydrochloride-polymyxin B sulfate vaginal tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride-polymyxin B sulfate vaginal tablets are tablets composed of oxytetracycline hydrochloride and polymyxin B sulfate, with one or more suitable diluents, binders, lubricants, and preservatives. Each tablet contains 100 milligrams of oxytetracycline and 100,000 units of polymyxin B. The moisture content is not more than 3.0 percent. The oxytetracycline hydrochloride used conforms to the standards prescribed by § 446.67a(a)(1) (i), (vi), (vii), (viii), and (ix). The polymyxin B sulfate used conforms to the standards prescribed by § 448.30a(a)(1) (i), (v), (vi), (vii), and (ix) of this chapter. Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline hydrochloride used in making the batch for potency, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, moisture, pH, residue on ignition, and identity.

(*c*) The batch for oxytetracycline content, polymyxin content, and moisture.

(ii) Samples required:

(*a*) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The batch: A minimum of 30 tablets.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Oxytetracycline content.* Proceed as directed in § 446.65a(b)(1)(i) or (ii), except prepare the sample as follows: Place a representative number of tablets in a glass blending jar and blend 3 to 5 minutes with an appropriate volume of 0.1*N* hydrochloric acid to give a stock solution of convenient concentration. Its content of oxytetracycline is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of oxytetracycline that it is represented to contain.

(ii) *Polymyxin content.* Proceed as directed in § 446.567c(b)(1)(ii) (*a*) or (*b*), using the finely powdered material from a representative number of tablets. Its content of polymyxin is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of units of polymyxin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) of this chapter.

Subpart H—Rectal Dosage Forms

§ 446.710 Chlortetracycline suppositories (chlortetracycline hydrochloride suppositories); tetracycline hydrochloride suppositories.

The requirements for certification and the tests and methods of assay for chlortetracycline suppositories (chlortetracycline hydrochloride suppositories); tetracycline hydrochloride suppositories are described in § 446.610.

Subpart I—[Reserved]

Subpart J—Certain Other Dosage Forms

§ 446.910 Chlortetracycline hydrochloride impregnated surgical silk.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chlortetracycline hydrochloride impregnated surgical silk is surgical silk that is impregnated with crystalline chlortetracycline hydrochloride. Its content of chlortetracycline hydrochloride is not less than 1.5 percent of the total weight of the suture. It is sterile. Its moisture content is not more than 4 percent. The chlortetracycline hydrochloride used conforms to the standards prescribed by § 446.10a(a)(1), except § 446.10a(a)(1)(ii), (iv) and (v). The surgical silk used conforms to the standards prescribed therefor by the U.S.P. for nonabsorbable surgical sutures.

(2) *Packaging.* The structures shall be packaged in tight containers as defined by the U.S.P. The immediate containers shall be so sealed that the contents cannot be used without destroying the seal and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused that are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded. Each immediate container may contain a suitable sterile surgical needle.

(3) *Labeling.* Each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the outside wrapper or container and the immediate container:

(*a*) The batch mark.

(*b*) The number of milligrams of chlortetracycline hydrochloride per suture.

(c) The length, size, and type of silk contained in the package.

(d) The statement "Expiration date ----------", the blank being filled in with the date that is 24 months after the month during which the batch was certified.

(e) The statement "Caution: Federal law prohibits dispensing without prescription".

(ii) [Reserved]

(iii) On the circular or other labeling within or attached to the package, if it is packaged for dispensing, adequate directions and warnings for its use by practitioners licensed by law to administer such drug.

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless it was previously submitted) the date on which the latest assay of the chlortetracycline hydrochloride used in making such batch was completed, the potency of each gram of the surgical silk, the date on which the latest assay of the batch was completed, and a statement that the surgical silk used in making the batch conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided in paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request results of the tests and assays listed after each of the following, made by him on an accurately representative sample of:

(a) The batch: Average potency per suture, moisture, and sterility.

(b) The chlortetracycline hydrochloride used in making the batch: Potency, toxicity, moisture, pH, and crystallinity.

(iii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(a) The batch:

(1) For all tests except sterility: 1 package for each 5,000 packages in the batch, but in no case less than 20 packages.

(2) For sterility testing: 20 packages. Such samples shall be collected by taking single packages at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(b) The chlortetracycline used in making the batch: 10 packages, each containing approximately equal portions of not less than 60 milligrams, packaged in accordance with the requirements of § 446.10a(a)(2).

(c) In case of an initial request for certification: 1 package consisting of approximately 1.0 gram of the surgical silk used.

(iv) The result referred to in paragraph (a)(4)(ii)(b) of this section and the sample referred to in paragraph (a)(4)(iii)(b) of this section are not required if such result or sample has been previously submitted.

(b) *Tests and methods of assay*—(1) *Potency.* Use not less than five sutures. Remove the needles, and place sutures in a glass-stoppered Erlenmeyer flask containing 50 milliliters of 0.1*N* hydrochloric acid and allow to stand overnight, with frequent agitation, at room temperature. Remove a suitable aliquot and further dilute in 0.1*M* phosphate buffer, pH 4.5, to the reference concentration, and proceed as directed in § 446.10a(b)(1)(viii). The potency is satisfactory if it contains not less than 85 percent of the number of milligrams of chlortetracycline hydrochloride per suture that it is represented to contain.

(2) *Sterility.* Using individual sutures, proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(2) of that section.

(3) *Moisture.* Using 10 sutures, proceed as directed in § 440.80a(b)(5) of this chapter.

PART 448—PEPTIDE ANTIBIOTICS

Subpart A—Bulk Drugs

Sec.
448.10a Sterile bacitracin.
448.13 Zinc bacitracin (bacitracin zinc salt).
448.13a Sterile zinc bacitracin.
448.15a Sterile capreomycin sulfate.
448.20a Sterile colistimethate sodium.
448.21 Colistin sulfate.
448.25 Gramicidin.
448.30a Sterile polymyxin B sulfate.
448.75 Tyrothricin.
448.80a Sterile viomycin sulfate.

Subpart B—Oral Dosage Forms

448.110 Bacitracin oral dosage forms.
448.110a Bacitracin tablets; zinc bacitracin tablets; bacitracin suppositories; zinc bacitracin suppositories (if they are represented for vaginal use).
448.110b Bacitracin-polymyxin tablets.
448.121 Colistin sulfate for oral suspension.

Subpart C—Injectable Dosage Forms

448.210 Sterile bacitracin.
448.215 Sterile capreomycin sulfate.
448.220 Colistimethate sodium injectable dosage forms.
448.220a Sterile colistimethate sodium.
448.220b Colistimethate sodium for injection.
448.280 Sterile viomycin sulfate.

Subpart D—Ophthalmic Dosage Forms

448.310 Bacitracin ophthalmic dosage forms.
448.310a Bacitracin ophthalmic.
448.310b Sterile bacitracin-neomycin sulfate-polymyxin B sulfate ophthalmic ointment; sterile bacitracin-neomycin sulfate-polymyxin B sulfate-hydrocortisone acetate ophthalmic ointment.
448.310c Bacitracin ointment; zinc bacitracin ointment.
448.313 Sterile zinc bacitracin-neomycin sulfate-polymyxin B sulfate ophthalmic ointment.
448.321 Colistin sulfate for ophthalmic solution.

Subpart E—Otic Dosage Forms

448.410 Bacitracin-neomycin undecylenate otic drops.
448.421 Colistin sulfate-neomycin sulfate-thonzonium bromide-hydrocortisone acetate otic suspension.
448.430 Polymyxin B sulfate otic solution.

Subpart F—Dermatologic Dosage Forms

448.510 Bacitracin dermatologic dosage forms.
448.510a Bacitracin ointment; zinc bacitracin ointment.
448.510b Bacitracin-tyrothricin ointment.
448.510c Bacitracin-polymyxin ointment; zinc bacitracin-polymyxin ointment.
448.510d Bacitracin-neomycin topical ointment; zinc bacitracin-neomycin topical ointment.
448.510e Bacitracin - polymyxin - neomycin ointment.
448.510f Bacitracin - neomycin - polymyxin powder topical; zinc bacitracin-neomycin-polymyxin powder topical.

Subpart G—Vaginal Dosage Forms

448.610 Bacitracin tablets; zinc bacitracin tablets; bacitracin suppositories; zinc bacitracin suppositories (if they are represented for vaginal use).

Subparts H–I—[Reserved]

Subpart J—Certain Other Dosage Forms

448.910 Bacitracin for prescription compounding.
448.913 Zinc bacitracin for prescription compounding.
448.930 Polymyxin B sulfate in certain other dosage forms.
448.930a Polymyxin B sulfate for prescription compounding.
448.930b Sterile polymyxin B sulfate-benzalkonium chloride urethral lubricant.

AUTHORITY: Sec. 507, 59 Stat. 463, as amended (21 U.S.C. 357).

Subpart A—Bulk Drugs

§ 448.10a Sterile bacitracin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Bacitracin is a white to brown, neutral water-soluble polypeptide. It is so purified and dried that:

(i) Its potency is not less than 40 units per milligram, except that if it is packaged for dispensing its potency is not less than 50 units per milligram.

(ii) It is sterile.

(iii) It is nontoxic.

(iv) It is nonpyrogenic.

(v) Its moisture content is not more than 5 percent.

(vi) Its pH in aqueous solution of 10,000 units per milliliter is not less than 5.5 and not more than 7.5.

(vii) Its ash content is not more than 3.0 percent.

(viii) If it is intended for systemic medication, its heavy metals content is not more than 30 parts per million.

(2) *Packaging.* In all cases the immediate containers shall be tight containers as defined by the U. S. P., shall be sterile at the time of filling and closing, shall be so sealed that the contents cannot be used without destroying the seal, and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so

caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded. In case it is packaged for dispensing, it shall be in immediate containers of colorless transparent glass, closed by a substance through which a hypodermic needle may be introduced and withdrawn without removing the closure or destroying its effectiveness; each such container shall contain not more than 60,000 units and may be packaged in combination with a container of the solvent, water for injection U. S. P., physiological salt solution U. S. P., or with a container of an aqueous solution of a suitable local anesthetic.

(3) Labeling—(i) *It is packaged for dispensing.* In addition to the labeling requirements prescribed by §1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its label or labeling, as hereinafter indicated, the following:

(*a*) An expiration date prescribed by § 432.5(a)(3) of this chapter.

(*b*) On the outside wrapper or container, if it is packaged for dispensing the statement "Store in refrigerator not above 15° C. (59° F.)" or "Store below 15° C. (59° F.)".

(*c*) On the circular or other labeling within or attached to the package, the conditions under which such solutions should be stored, including a reference to their instability when stored under other conditions; and the statement "Sterile solution may be kept in refrigerator for 1 week without significant loss of potency".

(ii) *It is packaged solely for manufacturing use and/or repacking.* Each package shall bear on its outside wrapper or container and the immediate container the following:

(*a*) The number of units of bacitracin per milligram or per gram and the number of grams or kilograms in the immediate container.

(*b*) The statement "Caution: Federal law prohibits dispensing without prescription".

(*c*) The statement "For manufacturing use", "For repacking", or "For manufacturing use or repacking".

(*d*) The information required by paragraph (a)(3)(i) (*a*) and (*b*) of this section.

(4) *Request for certification, check tests and assays; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch of bacitracin shall submit with his request a statement showing the batch mark, the number of packages of each size in the batch, the number of units in each package, and (unless it was previously submitted) the date on which the latest assay of the drug comprising the batch was completed. Such request shall be accompanied or followed by the results of tests and assays made by him on the batch for potency, sterility, toxicity, pyrogens, moisture, pH, ash content, and heavy metals. If such batch or any part thereof is to be packaged with a solvent such request shall also be accompanied by a statement that such solvent conforms to the requirements prescribed therefor by this paragraph.

(ii) If such batch is packaged for dispensing, such person shall submit with his request an accurately representative sample of the batch, consisting of the following:

(*a*) For all tests except sterility: One immediate container for each 5,000 immediate containers in such batch, but in no case less than nine immediate containers. If a sample of such batch has not been previously submitted in accordance with paragraph (a)(4)(iii) or (iv) of this section, such person shall submit one additional package containing 1.0 gram of the batch.

Such samples shall be collected by taking single immediate containers at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(*b*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(iii) If such batch is packaged for repacking or for use as an ingredient in the manufacture of another drug, such person shall submit with his request an accurately representative sample of the batch, consisting of the following:

(*a*) For all tests except sterility: 6 packages.

(*b*) For sterility testing: 20 packages.

Each such package shall contain approximately 0.5 gram, taken from a different part of such batch, and each shall be packaged in accordance with the requirements of paragraph (a)(2) of this section.

(iv) In connection with contemplated requests for certification of repacked batches or batches of another drug in the manufacture of which it is to be used, the manufacturer of a batch which is to be so repacked or used may request the Commissioner to make check tests and assays on a sample of such batch, taken as prescribed by paragraph (a)(4)(iii) of this section. From the information required by paragraph (a)(4)(i) of this section may be omitted results of tests and assays not required for the batch when used in such other drug. The Commissioner shall report to such manufacturer results of such check tests and assays as are so requested.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Plate assay.* Using the standard curve technique proceed as directed in § 440.80a(b)(1)(viii) of this chapter, with the following exceptions:

(*a*) Dry the working standard (obtained from the Food and Drug Administration) for 3 hours at 60° C. under 5 millimeters or less pressure. Weigh a sufficient amount to make a convenient stock solution and dilute in 1 percent phosphate buffer, pH 6.0. The stock solution when refrigerated may be used for two weeks. The stock solution may also be served for at least two months by freezing in small aliquots. Each aliquot should be sufficient for 1 day's use only. Make all dilutions of the stock solution for the assay with 1 percent phosphate buffer.

(*b*) Dissolve the sample to be tested in 1-percent phosphate buffer and make dilutions with the same solvent to one unit per milliliter (estimated).

(*c*) The test organism is either *Micrococcus flavus* (ATCC 10240) or *Sarcina subflava* (ATCC 7468),[1] both of which are maintained at refrigerator temperature on slants of nutrient agar prepared as directed in § 440.80a(b)(1)(ii)(*a*) of this chapter. Inoculate a Roux bottle containing this agar from a stock slant of the organism and incubate 18 hours at 32° C.–35° C. Wash off the growth in 25 milliliters of sterile sodium chloride solution. If an aliquot of this bulk suspension, when diluted 1:50 in sodium chloride solution, gives 75 percent light transmission, if it is *Micrococcus flavus* (ATCC 10240),[1] or 50 percent light transmission, if it is *Sarcina subflava* (ATCC 7468)[1] in a suitable photoelectric colorimeter equipped with a filter having a wavelength of 6,500 Angstrom units, the bulk suspension is satisfactory for use. It may be necessary to adjust the bulk suspension by dilution so that an aliquot of the adjusted suspension diluted 1:50 gives 75 percent light transmission, if it is *Micrococcus flavus* (ATCC 10240)[1] or 50 percent light transmission if it is *Sarcina subflava* (ATCC 7468).[1] (The adjusted bulk suspension only, and not the 1:50 dilution of it, is used in preparing the seed layer.) Add 0.3 to 0.5 milliliter of the adjusted bulk suspension to 100 milliliters of agar that has been melted and cooled to 48° C.

(ii) *Turbidimetric assay.* In lieu of the plate-assay method described above, the sample may be assayed for potency by the following turbidimetric method:

(*a*) *Test culture and media.* The test organism is *Staphylococcus aureus* (ATCC 10537),[1] which is maintained at refrigerator temperature on slants of nutrient agar prepared as directed in 440.80a(b)(1)(ii)(*a*) of this chapter. Inoculate a Roux bottle containing this agar from a stock slant of the organism and incubate 18 hours at 32° C.–35° C. Wash off the growth in 50 milliliters of sterile sodium chloride solution. If an aliquot of the bulk suspension, when diluted 1:10 in sodium chloride solution, gives 20 percent light transmission in a suitable photoelectric colorimeter equipped with a filter having a wavelength of 6,500 Angstrom units, the bulk suspension is ready for use. It may become necessary to adjust the bulk suspension by dilution so that an aliquot of the adjusted suspension diluted 1:10 gives 20 percent light transmission. (The adjusted suspension only, and not the 1:10 dilution, is used in preparing the daily inoculum.) Prepare the daily inoculum by adding approximately 0.2 milliliter of the adjusted suspension to each 100 milliliters of refrigerated broth.

(*b*) *Working standard solutions.* Dilute the working standard to 10 units

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

per milliliter of bacitracin in 1 percent phosphate buffer. Further dilute this to make solutions containing 0.256, 0.320, 0.400, 0.500, and 0.625 units per milliliter. These solutions are used for preparing the standard curve and may be held under refrigeration for 1 week. Add 1.0 milliliter of each of these working standard solutions to each of three 16 millimeter x 125 millimeter tubes (outside dimension).

(c) *Preparation of sample.* Dilute the sample under test to 0.400 unit per milliliter (estimated). Add 1.0 milliliter of the diluted sample to each of three 16 millimeter x 125 millimeter tubes (outside dimension). Add 9.0 milliliters of the "daily" inoculum described in paragraph (b) (ii) (*a*) of this section to each tube of the standard and unknown series and place immediately in a 37° C. water bath for 4 hours. After incubation, add four drops of formaldehyde to each tube, and estimate the turbidity of each in a photoelectric colorimeter, using a broad-band filter having a wavelength of 5300 Angstrom units.

(d) *Estimation of potency.* Average the three colorimeter readings at each standard level. Plot the average absorbance figures of the standard on semilog graph paper, employing units per tube as the logarithmic scale and absorbance as the arithmetic scale. Connect the points with a straightedge. Average the sample readings and read in units per tube from the curve. Units per tube divided by 0.400 x 100 will give percent potency of the sample.

(iii) *Bacitracin content.* The potency of bacitracin is satisfactory when assayed by the methods described in this paragraph if the immediate containers contain not less than 85 percent and, if it is intended for systemic medication, not more than 115 percent of the number of units they are represented to contain.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e) (1) of that section.

(3) *Pyrogens.* Proceed as directed in § 440.80a(b) (3) of this chapter, using as a test dose 1.0 milliliter per kilogram of a solution containing 300 units per milliliter. Use physiological salt solution as the diluent.

(4) *Toxicity.* Inject intravenously each of five mice within the weight range of 18–25 grams with 0.5 milliliter of a solution of the sample prepared by diluting with sterile physiological salt solution to 200 units per milliliter. The injection should be made over a period of not more than 5 seconds. If no animal dies within 48 hours, the sample is nontoxic. If one or more animals die within 48 hours, repeat the test, using for each test five or more previously unused mice weighing 20 grams (±0.5 gram) each; if the total deaths within 48 hours is no greater than 10 percent of the total number of animals tested, including the original test, the sample is nontoxic.

(5) *Moisture.* Proceed as directed in § 440.80a(b) (5) (i) of this chapter.

(6) *pH.* Proceed as directed in § 440.80a(b) (5) (ii) of this chapter, using a solution containing 10,000 units per milliliter.

(7) *Ash content.* Place approximately 1 gram of the sample, accurately weighed, in a tared porcelain crucible and carefully ignite at a low temperature until thoroughly charred. The crucible may be loosely covered with a porcelain lid during the charring. Add to the contents of the crucible 2 milliliters of nitric acid and 5 drops of sulfuric acid, and cautiously heat until white fumes are evolved, then ignite, preferably in a muffle furnace, at 500° C. to 600° C. until the carbon is all burned off. Cool the crucible in a desiccator and weigh. From the weight of residue obtained, calculate the sulfated ash content.

(8) *Heavy metals content.* Using the sulfated ash obtained in paragraph (b) (7) of this section, proceed as directed in the U.S.P. Heavy Metals Test, Method II, starting with the words "Cool, add 2 ml. of hydrochloric acid * * *."

§ 448.13 Zinc bacitracin (bacitracin zinc salt).

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Zinc bacitracin is the zinc salt of a kind of bacitracin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 40 units per milligram.

(ii) It is nontoxic.

(iii) Its moisture content is not more than 5 percent.

(iv) Its pH is not less than 6.0 and not more than 7.5.

(v) Its zinc content is not more than 10 percent by weight on a moisture-free basis.

(2) *Packaging.* In all cases the immediate containers shall be tight containers as defined by the U.S.P. The composition of the immediate containers shall be such as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused that are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* Each package of zinc bacitracin shall bear on its outside wrapper or container and the immediate container:

(i) The batch mark.

(ii) The number of units of bacitracin activity per milligram of zinc bacitracin and the number of grams of the drug in the immediate container.

(iii) The statement "Expiration date ----------", the blank being filled in with the date which is 24 months after the month during which the batch was certified, except that the blank may be filled in with the date that is 30 months, 36 months, 42 months, 48 months, 54 months, or 60 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time such drug as prepared by him complies with the standards prescribed therefor by paragraph (a) (1) of this section.

(iv) The statement "For use only in the manufacture of nonparenteral drugs".

(v) The statement "Caution: Federal law prohibits dispensing without prescription".

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in the batch, the number of units of bacitracin activity per milligram of zinc bacitracin, the zinc content, and the total number of grams of zinc bacitracin in each package. Such request shall be accompanied or followed by the results of tests and assays made by him on the batch for potency, toxicity, moisture, pH, and zinc content.

(ii) Such person shall also submit with his request an accurately representative sample of the batch, consisting of 5 packages each containing approximately 1.0 gram taken from a different part of such batch, and each shall be packaged in accordance with the requirements of paragraph (a) (2) of this section.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed for bacitracin in § 436.105 of this chapter, except add to each standard response line concentration sufficient 0.01*N* hydrochloric acid to yield the same ratio of 0.01*N* hydrochloric acid to 1 percent potassium phosphate buffer, pH 6.0 (solution 1) as present in the sample solution diluted to the reference concentration. Prepare the sample for assay as follows: Dissolve an accurately weighed sample (usually 25 to 35 milligrams) in sufficient 0.01*N* hydrochloric acid to give a bacitracin concentration of 100 units per milliliter (estimated). Further dilute with solution 1 to the reference concentration of 1.0 unit of bacitracin per milliliter (estimated).

(2) *Toxicity.* Administer orally, by means of a cannula or other suitable device, to each of 5 mice within the weight range of 18–25 grams, 0.5 milliliter of a water suspension of the drug containing 4,000 units per milliliter. If no animal dies within 48 hours, the sample is nontoxic. If one or more animals die within 48 hours, repeat the test, using for each test five or more previously unused mice weighing 20 grams (±0.5 grams) each; if the total deaths within 48 hours is no greater than 10 percent of the total number of animals tested, including the original test, the sample is nontoxic.

(3) *Moisture.* Proceed as directed in § 440.80a(b) (5) (i) of this chapter.

(4) *pH.* Prepare a saturated solution, using approximately 100 milligrams of the sample per milliliter, and proceed as directed in § 440.80a(b) (5) (ii) of this chapter.

(5) *Zinc content—*(i) *Reagents—*(*a*) *Aqueous working solution.* Dissolve 3.11 grams of the zinc oxide in sufficient 1*N* HCl and dilute to 250 milliliters with water. Remove a suitable aliquot and

dilute with water to obtain 0.1 percent solution.

(b) *Alkaline buffer solution.* Mix one part 10*N* ammonium hydroxide and 4.5 parts of 1*N* ammonium chloride.

(c) *1 percent phenolphthalein in absolute alcohol.*

(d) *Ethylenediaminetetraacetic acid sodium working solution.* Dissolve 3.72 grams of ethylenediaminetetraacetic acid sodium in 100 milliliters of water. Remove an aliquot and prepare a 1:25 dilution using water as the diluent.

(e) *1-(1-Hydroxy-2-naphthylazo)-5-nitro-2-naphthol-4-sulfonic acid sodium salt solution.* Prepare daily a 1 percent solution in alkaline buffer.

(ii) *Preparation of sample.* Using 2 milliliters of 10 percent acetic acid and 5 milliliters of water, wash an amount of the sample containing approximately 2 milligrams of zinc (usually 40 milligrams to 50 milligrams) into a titration flask. Add 1 drop of phenolphthalein solution. Add 1*N* NaOH, dropwise, until a heavy precipitate of bacitracin appears and a faint pink color persists.

(iii) *Preparation of standard.* Place 2-milliliter aliquots of the 0.1 percent working solution into two titration flasks, respectively, and add one drop of phenolphthalein solution.

(iv) *Procedure.* To all titration flasks (standard and sample) add 2 milliliters of alkaline buffer solution, 50 milliliters of water, and 0.1 milliliter of 1-(1-hydroxy-2-naphthylazo)-5-nitro-2-naphthol-4-sulfonic acid sodium salt indicator solution. Titrate with ethylenediaminetetraacetic acid working solution from a burgundy-red color to a blue endpoint.

(v) *Calculation.* From the standards, calculate the milligram equivalent of the ethylenediaminetetraacetic acid solution per milliliter (F).

$$\text{Percent zinc} = \frac{F \times \text{milliliters of ethylenediamine-tetraacetic acid (sample)} \times 100}{\text{Weight of samples in milligrams (on anhydrous basis)}}$$

§ 448.13a Sterile zinc bacitracin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile zinc bacitracin is the zinc salt of a kind of bacitracin or a mixture of two or more such salts. It is so purified and dried that:

(i) It contains not less than 40 units of bacitracin per milligram.

(ii) It is sterile.

(iii) It passes the safety test.

(iv) Its loss on drying is not more than 5.0 percent.

(v) Its pH is not less than 6.0 and not more than 7.5.

(vi) Its zinc content is not more than 10 percent by weight on a moisture-free basis.

(2) *Labeling.* In addition to the labeling requirements of § 432.5 of this chapter, each package shall bear on the outside wrapper or container and the immediate container the statement "For use in the manufacture of topical drugs only".

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, safety, loss on drying, pH, and zinc content.

(ii) Samples required:

(a) For all tests except sterility: Six packages, each containing approximately 1.0 gram.

(b) For sterility testing: 20 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.*—Proceed as directed for bacitracin in § 436.105 of this chapter, except add to each standard response line concentration sufficient 0.01*N* hydrochloric acid to yield the same ratio of 0.01*N* hydrochloric acid to 1 percent potassium phosphate buffer, pH 6.0 (solution 1) as present in the sample solution diluted to the reference concentration. Prepare the sample for assay as follows: Dissolve an accurately weighed sample (usually 25 to 35 milligrams) in sufficient 0.01*N* hydrochloric acid to give a bacitracin concentration of 100 units per milliliter (estimated). Further dilute with solution 1 to the reference concentration of 1.0 unit of bacitracin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid F in lieu of diluting fluid A.

(3) *Safety.* Proceed as directed in § 436.33 of this chapter.

(4) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using a saturated solution (approximately 100 milligrams of the sample per milliliter).

(6) *Zinc content*—(i) *Reagents*—(a) *Aqueous working solution.* Dissolve 3.11 grams of the zinc oxide in sufficient 1*N* HCl and dilute to 250 milliliters with water. Remove a suitable aliquot and dilute with water to obtain a 0.1 percent solution.

(b) *Alkaline buffer solution.* Mix one part 10*N* ammonium hydroxide and 4.5 parts of 1*N* ammonium chloride.

(c) *One percent phenolphthalein in absolute alcohol.*

(d) *Ethylenediaminetetraacetic acid sodium working solution.* Dissolve 3.72 grams of ethylenediaminetetraacetic acid sodium in 100 milliliters of water. Remove an aliquot and prepare a 1:25 dilution using water as the diluent.

(e) *1-(1-hydroxy-2-naphthylazo)-5-nitro-2-naphthol-4-sulfonic acid sodium salt indicator solution.* Prepare daily a 1 percent solution in alkaline buffer.

(ii) *Preparation of sample.* Using 2 milliliters of 10 percent acetic acid and 5 milliliters of water, wash an amount of the sample containing approximately 2 milligrams of zinc (usually 40 milligrams to 50 milligrams) into a titration flask. Add 1 drop of phenolphthalein solution. Add 1*N* NaOH, dropwise, until a heavy precipitate of bacitracin appears and a faint pink color persists.

(iii) *Preparation of standard.* Place 2-milliliter aliquots of the 0.1 percent working solution into two titration flasks, respectively, and add one drop of phenolphthalein solution.

(iv) *Procedure.* To all titration flasks (standard and sample) add 2 milliliters of alkaline buffer solution, 50 milliliters of water, and 0.1 milliliter of 1-(1-hydroxy-2-naphthylazo)-5-nitro-2-naphthol-4-sulfonic acid sodium salt indicator solution. Titrate with ethylenediaminetetraacetic acid working solution from a burgundy-red color to a blue endpoint.

(v) *Calculation.* From the standards, calculate the milligram equivalent of the ethylenediaminetetraacetic acid solution per milliliter (F):

$$\text{Percent zinc} = \frac{F \times \text{milliliters of ethylenediaminetetraacetic acid (sample)} \times 100}{\text{Weight of samples in milligrams (on anhydrous basis)}}$$

§ 448.15a Sterile capreomycin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile capreomycin sulfate is the amorphous sulfate salt of capreomycin. It is a white or essentially white powder. Capreomycin has been separated chromatographically into components designated capreomycins Ia, Ib, IIa, and IIb. Each component has been partially characterized according to its type and amino acid content. Capreomycin Ia contains serine and no alanine. Capreomycin Ib contains alanine and no serine. Capreomycin I is a mixture of capreomycins Ia and Ib. It is so purified and dried that:

(i) Its potency is not less than 700 micrograms and not more than 1,050 micrograms of capreomycin per milligram on an "as is" basis. If it is packaged for dispensing, its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of capreomycin that it is represented to contain.

(ii) It is sterile.

(iii) It passes the safety test.

(iv) It is nonpyrogenic.

(v) In contains no histamine nor histamine-like substance.

(vi) Its loss on drying is not more than 10 percent.

(vii) Its pH in an aqueous solution containing 30 milligrams per milliliter (or if packaged for dispensing, after reconstitution as directed in the labeling) is not less than 4.5 and not more than 7.5.

(viii) Its capreomycin I content is not less than 90 percent of the total capreomycins.

(ix) Its residue on ignition is not more than 3 percent.

(x) Its heavy metals content is not more than 30 parts per million.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, safety, pyrogens, histamine, loss on drying, pH, capreomycin I content, residue on ignition, and heavy metals.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 500 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to give a stock solution of convenient concentration; also, if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with sterile distilled water to give a stock solution of convenient concentration. Further dilute the stock solution with sterile distilled water to the reference concentration of 100 micrograms of capreomycin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Safety.* Proceed as directed in § 436.33 of this chapter.

(4) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 10 milligrams per milliliter.

(5) *Histamine.* Proceed as directed in § 436.35 of this chapter.

(6) *Loss on drying.* Proceed as directed in § 436.200(e) of this chapter.

(7) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 30 milligrams per milliliter; however, if it is packaged for dispensing, use the solution obtained after reconstituting the drug as directed in the labeling.

(8) *Capreomycin I content*—(i) *Equipment*—(*a*) *Sheet (chromatographic).* Whatman No. 1 filter paper for chromatography 20 x 50 centimeters.

(*b*) *Chamber (chromatographic).* Square glass chromatography jar, 30 x 30 x 60 centimeters, designed for descending chromatography. The bottom of the tank is filled with 1.5 inches of a mixture of 70 percent *n*-propyl alcohol and 30 percent distilled water (v/v) and allowed to equilibrate for 2 days. The mobility of the capreomycin factors, capreomycin I and capreomycin II, depends to a large extent upon the amount of water vapor present in the chromatographic chamber. The mobility can be restricted by using more *n*-propyl alcohol and less water in the equilibrating solvent, or it can be increased by raising the water content. The R_f value (the distance traveled by a particular antibiotic factor divided by the distance traveled by the solvent front) should be approximately 0.50 for capreomycin I and approximately 0.60 for capreomycin II.

(ii) *Preparation of solutions*—(*a*) *0.1N citrate buffer, pH 6.2.* Dissolve 21.0 grams of citric acid monohydrate in 1 liter of distilled water. Adjust the pH to 6.2 with 50 percent aqueous sodium hydroxide.

(*b*) *Developing solvent.* Mix *n*-propyl alcohol, distilled water, triethylamine, and glacial acetic acid in volumetric proportions of 75:33:8:8, respectively.

(iii) *Preparation of the capreomycin sample solution.* Dissolve approximately 200 milligrams of the sample, accurately weighed, with distilled water in a 10-milliliter volumetric flask. Dilute to volume with distilled water. This sample should be refrigerated when not in use.

(iv) *Preparation of the chromatogram.* Use separate sheets for each capreomycin sample solution and for blanks without sample application. Evenly apply a 100-microliter aliquot of the capreomycin sample solution to the origin line of a sheet. A U-shaped glass rod is placed under the chromatogram during spotting. Dry the streak thoroughly with warm air. Place the sample sheets and a blank sheet in the chamber and develop them in a descending manner for 16 hours. Remove the sheets from the chamber and air dry for about 1 hour.

(v) *Processing the chromatogram.* Examine each sheet under short-wavelength (254 nanometers) ultraviolet light and locate the main streak (R_f approximately 0.5) and the preceding streak (capreomycin II, R_f approximately 0.6). Outline the main zone lightly with a pencil. Outline an area on the blank sheet approximately equal in size and in the same location as those outlined on the sample sheets. Cut the marked areas from the sheets and then cut them into approximately 1.5-centimeter squares. For each sheet, place the squares into a glass-stoppered 50-milliliter Erlenmeyer flask.

(vi) *Elution.* To each flask, add 10 milliliters of 0.1*N* citrate buffer, pH 6.2, and agitate on a reciprocating shaker for 1 hour. Filter each of the shaken solutions through Whatman No. 1 filter paper into separate 10-milliliter glass-stoppered Erlenmeyer flasks. Transfer 3 milliliters of each filtrate into separate 50-milliliter volumetric flasks and dilute to volume with distilled water.

(vii) *Capreomycin sample solution for direct measurement of absorbance.* Pipette 1.0 milliliter of the sample solution prepared as described in paragraph (b)(8)(iii) of this section into a 100-milliliter glass-stoppered volumetric flask. Dilute to volume with 0.1*N* citrate buffer, pH 6.2. Transfer 3.0 milliliters of this solution into a 50-milliliter volumetric flask and dilute to volume with distilled water.

(viii) *Absorbance measurement.* Using a suitable spectrophotometer, 1.0-centimeter quartz cells, and distilled water as the reference solvent, determine the absorbance of each eluate and of each sample solution at the absorption maximum at about 268 nanometers.

(ix) *Calculation of percent capreomycin I in samples.* Calculate as follows:

$$\text{Percent capreomycin I} = \frac{A_I - A_B}{A_s} \times 100,$$

where:

A_I = Absorbance of the eluate from the main zone of the sample sheet;

A_B = Absorbance of the eluate from the area of the blank sheet corresponding to the area of the capreomycin I of the sample sheet;

A_s = Absorbance of the capreomycin sample solution described in paragraph (b)(8)(vii) of this section.

If the assay of capreomycin I from the chromatogram is less than 90 percent of total capreomycins, repeat the procedure described in paragraph (b)(8)(iv), (v), (vi), (vii), and (viii) of this section two more times and at the same time determine the recovery of total capreomycins from the unchromatographed sheet as described in paragraph (b)(8)(x) of this section. The average of three valid assays should then be reported.

(x) *Recovery of total capreomycins from the unchromatographed sheet*—(*a*) *Procedure.* Evenly apply a 100-microliter aliquot of the capreomycin sample solution (prepared as described in paragraph (b)(8)(iii) of this section) to the origin line of a sheet. Dry the streak thoroughly with warm air. The paper is not chromatographed before elution. Cut the area containing the streak from the sheet and then cut into approximately 1.5-centimeter squares. Place the squares into a glass-stoppered 50-milliliter Erlenmeyer flask and proceed as directed in paragraph (b)(8)(vi), (vii), and (viii) of this section. Likewise, cut an equal-sized area from an untreated part of the sheet and cut it into approximately 1.5-centimeter squares. Place the squares in a glass-stoppered 50-milliliter Erlenmeyer flask and also proceed as directed in paragraph (b)(8)(vi), (vii), and (viii) of this section.

(b) *Calculation.* Calculate the recovery of total capreomycins as follows:

$$\text{Recovery of total capreomycins} = \frac{A_I - A_b}{A_s} \times 100,$$

where:

A_I = Absorbance of the eluate from the unchromatographed sheet;

A_b = Absorbance of the eluate from the unchromatographed blank sheet;

A_s = Absorbance of the capreomycin sample solution described in paragraph (b)(8)(vii) of this section.

To be a valid assay, the recovery of total capreomycins from the unchromatographed sheet must be 100±2 percent.

(9) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter, except ignite at 700° C.

(10) *Heavy metals.* Proceed as directed in § 436.208 of this chapter.

§ 448.20a Sterile colistimethate sodium.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Colistimethate sodium is the sodium salt of a kind of colistin methane sulfonate or a mixture of two or more such salts. It is a white to slightly yellow, odorless, fine powder which is freely soluble in water. It is so purified and dried that:

(i) Its potency is not less than 390 micrograms of colistin base equivalent per milligram. If it is packaged for dispensing, its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of colistin base equivalent that it is represented to contain.

(ii) It is sterile.

(iii) It passes the safety test.

(iv) It is nonpyrogenic.

(v) Its loss on drying is not more than 9.0 percent.

(vi) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 6.5 and not more than 9.0. If it is packaged for dispensing, its pH is not less than 6.0 and not more than 8.0 when reconstituted as directed in the labeling.

(vii) It gives a positive identity test for colistimethate sodium.

(viii) Its residue on ignition is not less than 24.8 percent and not more than 28.8 percent.

(ix) Its heavy metals content is not more than 30 parts per million.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, safety, pyrogens, loss on drying, pH, identity, residue on ignition, and heavy metals.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: 10 containers, each containing approximately 500 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 12 vials or if each vial contains less than 150 milligrams of colistimethate, a minimum of 60 vials.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: If the batch is packaged for repacking or for use in manufacturing another drug, dissolve an accurately weighed sample in 2 milliliters of sterile distilled water and further dilute with sufficient 10-percent potassium phosphate buffer, pH 6.0 (solution 6), to give a stock solution of convenient concentration. If it is packaged for dispensing, reconstitute as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if the container is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Further dilute the stock solution with solution 6 to the reference concentration of 1.0 microgram of colistin base equivalent per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 10 milligrams of colistin base equivalent per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this chapter.

(5) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using a 1-percent aqueous solution prepared in the following manner: Weigh accurately 0.5 gram of sample and transfer to a 125-milliliter Erlenmeyer flask. Add 50 milliliters of freshly boiled distilled water, stopper, and shake until the sample is in solution. Allow to stand for one-half hour before determining the pH. If it is packaged for dispensing, use the solution obtained after reconstituting the drug as directed in the labeling.

(7) *Identity.* To about 20 milligrams of sample, add 2.0 milliliters of pH 7.0 buffer (prepared by adding 29.63 milliliters of 1*N* sodium hydroxide to 50 milliliters of 1*M* potassium dihydrogen phosphate, adjusting to pH 7.0 if necessary, and diluting to 100 milliliters with distilled water) and 0.2 milliliter of a 0.5 percent aqueous triketohydrindene hydrate solution. When heated for about 2 minutes, no pink color results. To another 20 milligrams of sample, add 2.0 milliliters of diluted hydrochloric acid U.S.P., heat to boil, and boil gently for 2 minutes. Neutralize with 10 percent sodium hydroxide to approximately pH 7 (indicator paper), add 2.0 milliliters of pH 7.0 buffer and 0.2 milliliter of a 0.5 percent aqueous triketohydrindene hydrate solution. Heat over an open flame for 2 minutes. A purple color is produced.

(8) *Residue on ignition.* Proceed as directed in § 436.207(b) of this chapter.

(9) *Heavy metals.* Proceed as directed in § 436.208 of this chapter.

§ 448.21 Colistin sulfate.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Colistin sulfate is the white to slightly yellow, odorless sulfate salt of a kind of colistin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 500 micrograms of colistin per milligram.

(ii) It passes the safety test.

(iii) Its loss on drying is not more than 7.0 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 4.0 and not more than 7.0.

(v) It gives a positive identity test for colistin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b).

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, loss on drying, pH, and identity.

(ii) Samples required on the batch: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in 2 milliliters of sterile distilled water and further dilute with sufficient 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to give a stock solution of convenient concentration. Further dilute the stock solution with solution 6 to the reference concentration of 1.0 microgram of colistin per milliliter (estimated).

(2) *Safety.* Proceed as directed in § 436.33 of this chapter.

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Identity.* To about 20 milligrams of sample, add 2.0 milliliters of pH 7.0 buffer (prepared by adding 29.63 milliliters of 1*N* sodium hydroxide to 50 milliliters of 1*M* potassium dihydrogen phosphate, adjusting to pH 7.0 if necessary, and diluting to 100 milliliters with distilled water) and 0.2 milliliters of a 0.5 percent aqueous triketohydrindene hydrate solution, and bring to boil. A purple color is produced.

§ 448.25 Gramicidin.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Gramicidin is the white, or nearly white, odorless, crystalline compound of a kind of gramicidin or a mixture of two or more such compounds. It is so purified and dried that:

(i) It has a potency of not less than 900 micrograms of gramicidin per milligram.

(ii) It passes the safety test.

(iii) Its loss on drying is not more than 3 percent.

(iv) Its residue on ignition is not more than 1.0 percent.

(v) It does not melt below 229° C. after drying in vacuum at 60° C. for 3 hours.

(vi) When calculated on the anhydrous basis, the difference between the absorptivity value at the maximum occurring at 282 nanometers and the absorptivity value at the minimum occurring at 247 nanometers is 100±4 percent of the difference obtained with the gramicidin working standard.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, loss on drying, residue on ignition, melting point, identity, and crystallinity.

(ii) Samples required of the batch: Ten packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 95 percent ethyl alcohol to give a stock solution of convenient concentration. Further dilute the stock solution volumetrically with 95 percent ethyl alcohol to the reference concentration of 0.04 microgram of gramicidin per milliliter (estimated).

(2) *Safety.* Proceed as directed in § 436.33 of this chapter, except observe the mice for 4 days.

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(5) *Melting point.* Proceed as directed in § 436.209 of this chapter.

(6) *Identity.* Accurately weigh about 20 milligrams of the sample and dilute in ethyl alcohol to give a concentration of 0.05 milligram (estimated) of gramicidin per milliliter. Prepare a solution of the gramicidin working standard to contain 0.05 milligram per milliliter in ethyl alcohol. Using a suitable recording spectrophotometer with 1-centimeter cells, record the ultraviolet absorbance spectrum of each solution from 220 nanometers to 320 nanometers. The ultraviolet absorbance spectrum of the sample solution should compare qualitatively to that of the working standard solution. Determine the absorptivities of each at the maximum occurring at 282 nanometers and at the minimum occurring at 247 nanometers (the exact position of the maximum and minimum of the gramicidin working standard should be determined for the particular instrument used).

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

§ 448.30a Sterile polymyxin B sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Polymyxin B sulfate is the sulfate salt of a kind of polymyxin or a mixture of two or more such salts. It is a white to buff-colored powder. It is so purified and dried that:

(i) Its potency is not less than 6,000 units of polymyxin B per milligram, on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It is nontoxic.

(v) Its moisture content is not more than 7.0 percent.

(vi) Its pH in an aqueous solution containing 5 milligrams per milliliter is not less than 5.0 and not more than 7.5.

(vii) Its residue on ignition is not more than 5 percent.

(viii) If it is intended for systemic medication, its heavy metals content is not more than 100 parts per million.

(ix) It gives positive color identity tests for polymyxin.

(2) *Packaging.* In addition to the requirements of § 432.1 of this chapter, if it is packaged for dispensing and it is intended for intrathecal, intramuscular, or topical use, each immediate container shall contain 500,000 units.

(3) *Labeling.* In addition to the requirements of § 432.5 of this chapter, if the drug is packaged for dispensing its labeling shall bear the statement, "Caution: This drug should be given intramuscularly and/or intrathecally only to hospitalized patients so as to provide constant supervision by a physician".

(4) *Request for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, toxicity, moisture, pH, residue on ignition, heavy metals, and identity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use as an ingredient in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the drug is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 10 immediate containers plus one additional package containing 1 gram of the batch.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Cylinders (cups).* Use cylinders described in § 440.80a(b)(1)(i) of this chapter.

(ii) *Culture media.* Using ingredients that conform to the standards, if any, prescribed by the U.S.P. or N.F., make nutrient agar for carrying the test organism and for the seed and base layers:

(*a*) For carrying the test organism:

Peptone	6.0 gm.
Pancreatic digest of casein	4.0 gm.
Beef extract	1.5 gm.
Yeast extract	3.0 gm.
Dextrose	1.0 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 6.5 to 6.8 after sterilization.

(*b*) Base layer:

Pancreatic digest of casein	17.0 gm.
Papaic digest of soybean	3.0 gm.
Sodium chloride	5.0 gm.
Dipotassium phosphate	2.5 gm.
Dextrose	2.5 gm.
Agar	20.0 gm.
Distilled water, q.s.	1,000.0 ml.

Final pH 7.3 after sterilization.

(*c*) Seed layer: For the seed layer, make the following changes in the medium described in paragraph (b)(1)(ii)(*b*) of this section.

Agar	12.0 gm.
Polysorbate 80 (added after boiling)	10.0 gm.

Final pH 7.3 after sterilization.

In lieu of preparing the media from the individual ingredients as specified, they may be prepared from a dehydrated mixture which, when reconstituted with distilled water, has the same composition as such media. Minor modifications of the specified individual ingredients are permissible if the resulting media possess growth-promoting properties at least equal to the media described.

(iii) *Working standard.* Dry approximately 30 milligrams of the standard for 3 hours at 60° C. and a pressure of 5 millimeters or less. Determine accurately the dry weight, dissolve in 2.0 milliliters of distilled water, and then add sufficient 10 percent potassium phosphate buffer, pH 6.0, to make a 1,000 units per milliliter stock solution. This solution may be used for 2 weeks if kept in refrigerator.

(iv) *Preparation of sample.* Dissolve an accurately weighed sample in 2 milliliters of distilled water and dilute with sufficient 10 percent potassium phosphate buffer, pH 6.0, to give a stock solution of convenient concentration; and, if it is packaged for dispensing, reconstitute as directed in the labeling, remove an accurately measured representative portion with a suitable syringe fitted with a 22-gage needle, and appropriately dilute in 10 percent potassium phosphate buffer, pH 6.0, to give a stock solution of convenient concentration. Further dilute in 10 percent potassium phosphate buffer, pH 6.0, to give a final estimated concentration of 10 units per milliliter of polymyxin.

(v) *Preparation of test organism.* The test organism is *Bordetella bronchiseptica* (ATCC 4617)[1] which is maintained on slants of agar described in paragraph (b)(1)(ii)(*a*) of this section Wash the organism from the agar slant with 3 milliliters of sterile U.S.P. saline T.S. onto a large surface such as that provided by a Roux bottle containing 300 milliliters of agar described in paragraph (b)(1)(ii)(*a*) of this section. Spread the suspension of organisms over the entire agar surface with the aid of sterile glass beads. Incubate for 24 hours at 37° C. Wash the resulting growth from the agar surface with 50 milliliters of sterile U.S.P. saline T.S. Standardize the suspension, so that a 1:20 dilution will give 25 per-

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

cent light transmission, using a suitable photoelectric colorimeter with a 580-millimicron filter and a 13-millimeter diameter test tube as an absorption cell. Run test plates to determine the quantity of the 1:20 diluted suspension (usually 0.3 percent) that should be added to each 100 milliliters of agar to give clear, sharp zones of inhibition of appropriate size.

(vi) *Preparation of plates.* Add 21 milliliters of the agar prepared as described in paragraph (b) (1) (ii) (b) of this section to each Petri dish (20 millimeters by 100 millimeters). Distribute the agar evenly in the plates, cover with porcelain covers glazed on the outside, and allow it to harden. Melt a sufficient amount of the agar described in paragraph (b) (1) (ii) (c) of this section, cool to 48° C.–50° C., add the proper amount of test organism as described in paragraph (b) (1) (v) of this section, and mix thoroughly. Add 4 milliliters of this inoculated agar to each Petri dish. Distribute the agar evenly in the plates, and allow to harden. After the agar has hardened, place 6 cylinders on the agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius.

(vii) *Standard curve.* Prepare the daily standard curve by further diluting the 1,000 units per milliliter working standard stock solution in 10 percent potassium phosphate buffer, pH 6.0, to obtain concentrations of 6.4, 8.0, 10.0, 12.5, and 15.6 units per milliliter. Use three plates for the determination of each point on the curve, except the 10.0 units per milliliter concentration, a total of 12 plates. On each of three plates, fill three cylinders with the 10.0 units per milliliter standard, and the other three cylinders with the concentration under test. Thus, there will be thirty-six 10.0-unit determinations and nine determinations for each of the other points on the curve. After incubation, read the diameters of the circles of inhibition in the plates. Average the readings of the 10.0 units per milliliter and the readings of the points tested for each set of three plates, and average also all 36 readings of the 10.0 units per milliliter concentration. The average of the 36 readings of the 10.0 units per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 10 units per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 8.0 units per milliliter concentration, the average of the 36 readings of the 10-unit concentration is 20.0 millimeters and the average for the 10-unit concentration of this set of three plates is 19.8 millimeters, the correction is +0.2 millimeter. If the average reading of the 8.0-unit concentration of these same three plates is 19.0 millimeters, the corrected value is 19.2 millimeters. Plot these corrected values, including the average of the 10 units per milliliter concentration, on semilog paper, placing the concentration in units per milliliter on the logarithmic scale and the diameter of the zone of inhibition on the arithmetic scale. Draw the standard curve through these points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

L = Calculated zone diameter for the lowest concentration of the standard curve;

H = Calculated zone diameter of the highest concentration of the standard curve;

c = Average zone diameter of 36 readings of the 10 units per milliliter standard;

a, b, d, e = Corrected values for the 6.4, 8.0, 12.5, and 15.6 units per milliliter standard solutions, respectively.

Plot the values obtained for L and H and connect with a straight line.

(viii) *Assay.* Use three plates for each sample. Fill three cylinders on each plate with the 10 units per milliliter standard solution and three cylinders with the 10 units per milliliter (estimated) sample, alternating sample and standard. Incubate all plates, including those containing the standard curve, for 16 hours to 18 hours at 37° C. Measure the diameter of each circle of inhibition. To estimate the potency of the sample, average the zone readings of the standard and the zone readings of the sample on the three plates used. If the sample gives a larger zone size than the average of the standard, add the difference between them to the 10 units per milliliter zone on the standard curve. If the average sample value is lower than the standard value, subtract the difference between them from the 10 units per milliliter value on the curve. Read the potencies corresponding to these corrected values of zone size. If packaged for dispensing, its content of polymyxin is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of units of polymyxin that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e) (1) of that section.

(3) *Pyrogens.* Proceed as directed in § 440.80a(b) (3) of this chapter, except use as a test dose 1.0 milliliter per kilogram of solution containing 20,000 units per milliliter in sterile pyrogen-free U.S.P. saline T.S.

(4) *Toxicity.* Proceed as directed in § 440.80a(b) (4) of this chapter, using a test dose of 0.5 milliliter of a solution containing 1,200 units per milliliter in sterile U.S.P. saline T.S.

(5) *Moisture.* Proceed as directed in § 440.80a(b) (5) (i) of this chapter.

(6) *pH.* Proceed as directed in § 440.80a(b) (5) (ii) of this chapter, except use a solution containing 5 milligrams per milliliter.

(7) *Residue on ignition.* Proceed as directed in § 448.10a(b) (7).

(8) *Heavy metals content.* Proceed as directed in § 436.208 of this chapter.

(9) *Identity.* (i) To a solution of 2 milligrams of polymyxin sulfate in 5 milliliters of water add 0.5 milliliter of triketohydrindene solution (1:1,000) and 2 drops of pyridine, boil for 1 minute, and cool; a blue color develops.

(ii) To a solution of 2 milligrams of polymyxin sulfate in 5 milliliters of water, add 5 milliliters of sodium hydroxide solution (1:10), mix well, and add, dropwise, 5 drops of a cupric sulfate solution (1:100), mixing after the addition of each drop; a reddish-violet color is produced.

§ 448.75 Tyrothricin.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Tyrothricin is a white to brownish-white compound of a kind of tyrothricin or a mixture of two or more such compounds. It consists principally of gramicidin and tyrocidine. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms and not more than 1,400 micrograms of tyrothricin per milligram.

(ii) Its loss on drying is not more than 5 percent.

(iii) It gives a positive identity test for tyrothricin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, and identity.

(ii) Samples required: five packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 95 percent ethyl alcohol, U.S.P. XVIII or equivalent, to give a stock solution of convenient concentration. Further dilute the stock solution with 95 percent ethyl alcohol, U.S.P. XVIII or equivalent, to the reference concentration of 0.20 microgram of tyrothricin per milliliter (estimated). Average the absorbance values for the tyrothricin sample and read the gramicidin concentration from the gramicidin standard response line. Multiply by 5 to obtain the number of micrograms of tyrothricin in the sample.

(2) *Loss on drying.* Proceed as directed in § 436.200 (b) of this chapter.

(3) *Identity.* To 5 milliliters of *p*-dimethylaminobenzaldehyde (T.S.) add about 5 milligrams of tyrothricin. Shake well for 2 minutes; then add 2 drops of 0.1*M* sodium nitrite and 5 milliliters of water. A blue color is produced.

§ 448.80a Sterile viomycin sulfate.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Viomycin sulfate is the sulfate salt of viomycin. It is so purified and dried that:

(i) Its potency is not less than 700 micrograms of viomycin per milligrams, on an anhydrous basis. If it is packaged for dispensing, its potency is satisfactory

if it is not less than 90 percent and not more than 115 percent of the number of milligrams of viomycin that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It passes the safety test.

(v) It contains no histamine nor histamine-like substances.

(vi) Its loss on drying is not more than 5 percent.

(vii) Its pH in an aqueous solution containing 100 milligrams per milliliter is not less than 4.5 and not more than 7.0.

(viii) Its absorption maximum at approximately 268 nanometers does not vary more than ±3 nanometers from that of the viomycin working standard, similarly treated.

(ix) It is crystalline.

(2) *Packaging.* In addition to the requirements of § 432.1 of this chapter, if it is packaged for dispensing the viomycin content shall be 1 gram or 5 grams of viomycin per immediate container.

(3) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(4) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, safety, histamine, loss on drying, pH, identity, and crystallinity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: A minimum of 10 packages, each containing 300 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 12 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to give a stock solution of convenient concentration; and also if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Further dilute the stock solution with sterile distilled water to the reference concentration of 100 micrograms of viomycin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 10 milligrams of viomycin per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this chapter.

(5) *Histamine.* Proceed as directed in § 436.35 of this chapter.

(6) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(7) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams of viomycin per milliliter.

(8) *Identity*—(i) *Phosphate buffer solution.* Mix 296.3 milliliters of 0.1*N* sodium hydroxide with 500 milliliters of 0.05*M* potassium phosphate (6.805 grams of KH_2PO_4 per 1,000 milliliters of water) and dilute with sufficient water to make 1 liter.

(ii) *Procedure.* Dissolve the working standard and the sample to be tested in sufficient potassium phosphate buffer solution to give solutions containing 10 micrograms per milliliter. Measure the ultraviolet absorption of each solution in a suitable spectrophotometer. Compare the spectrum of the sample qualitatively with that of the standard.

(9) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

Subpart B—Oral Dosage Forms

§ 448.110 Bacitracin oral dosage forms.

§ 448.110a Bacitracin tablets; zinc bacitracin tablets; bacitracin suppositories; zinc bacitracin suppositories (if they are represented for vaginal use).

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Bacitracin tablets and zinc bacitracin tablets are tablets composed of bacitracin or zinc bacitracin, with or without one or more suitable and harmless buffer substances, diluents, binders, lubricants, colorings, and flavorings, and with or without a suitable amebicidal agent. The potency of each tablet is not less than 1,000 units nor more than 10,000 units. Its moisture content is not more than 5 percent. Tablets not exceeding 15 millimeters in diameter shall disintegrate within 1 hour. The bacitracin used conforms to the standards prescribed therefor by § 448.10a(a)(1), except § 448.10a(a)(1) (ii), (iv) and (viii). The zinc bacitracin used conforms to the requirements of § 448.13 (a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* Unless each tablet is enclosed in a foil or plastic film and such enclosure is a tight container as defined by the U.S.P., except the provision that it shall be capable of tight reclosure, the immediate container shall be a tight container as so defined. The immediate container may also contain a desiccant separated from the tablets by a plug of cotton or other like material. The composition of the immediate container, or of the foil or film enclosure, shall be such as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106 (b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its label and labeling, as hereinafter indicated, the following:

(i) On the outside wrapper or container and the immediate container, the statement "Expiration date _____", the blank being filled in with the date that is 12 months after the month during which the batch was certified, except that the blank may be filled in with the date that is 18 months, 24 months, 36 months, or 48 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time such drug as prepared by him complies with the standards prescribed by paragraph (a)(1) of this section.

(ii) If it contains an amebicidal agent, after the name "bacitracin tablets", or "zinc bacitracin tablets," wherever it appears, the words "with _____", in juxtaposition with such name, the blank being filled in with the established name of the amebicidal agent used.

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless it was previously submitted) the date on which the latest assay of the bacitracin or zinc bacitracin used in making such batch was completed, the number of units in each tablet, the quantity of each ingredient used in making the batch, the date on which the latest assay of the drug comprising such batch was completed, and a statement that each ingredient used in making the batch conforms to the requirements prescribed therefor, if any, by this section.

(ii) Except as otherwise provided in paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request results of the tests and assays listed after each of the following, made by him on an accurately representative sample of:

(*a*) The batch: Average potency per tablet, average moisture, and if required by paragraph (a)(1) of this section, disintegration time.

(*b*) The bacitracin or zinc bacitracin used in making the batch; potency, toxicity, moisture, pH, ash content if it is bacitracin, and zinc content if it is zinc bacitracin.

(iii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch:

(*1*) For potency and moisture: One tablet for each 5,000 tablets in the batch, but in no case less than 30 tablets, collected by taking single tablets throughout the entire time of tableting so that the quantities tableted during the intervals are approximately equal.

(2) For distintegration time: 6 tablets.

(*b*) The bacitracin used in making the batch; six packages, each containing approximately equal portions of not less than 500 milligrams each, packaged in accordance with the requirements of § 448.10a(a)(2).

(*c*) The zinc bacitracin used in making the batch; 6 packages, each containing approximately equal portions of not less than 1.0 gram, packaged in accordance with the requirements of § 448.13(a)(2).

(*d*) In case of an initial request for certification, each other substance used in making the batch; 1 package of each containing approximately 5 grams.

(iv) No result referred to in paragraph (a)(4)(ii)(*b*) of this section, and no sample referred to in paragraph (a)(4)(iii) (*b*), (*c*) or (*d*) of this section is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 448.10a(b)(1), except § 448.10a(b)(1)(iii), and in addition to the directions in § 448.10a(b)(1)(i)(*b*), proceed as follows: If it is bacitracin, place a representative sample (usually 5 tablets) in a blending jar containing 250 milliliters of 1-percent phosphate buffer, pH 6.0. If it is zinc bacitracin, use 250 milliliters of 0.01*N* HCl. If it is bacitracin, after blending for 1 minute with a high-speed blender, add 250 milliliters of buffer to the blender, and if it is zinc bacitracin, add 250 milliliters of 0.01*N* HCl to the blender. Blend again for 1 minute and make the proper estimated dilutions in 1-percent phosphate buffer, pH 6.0. If it is zinc bacitracin, in lieu of the directions in § 448.10a(b)(1)(i)(*a*) prepare the standard as follows. Using the stock solution described in § 448.10a(b)(1)(i)(*a*), make all dilutions for the assay so that the final dilution contains the same ratio of 1.01 *N* HCl to 1-percent phosphate buffer, pH 6.0, as the sample under test. The content of bacitracin, or zinc bacitracin is satisfactory if it contains not less than 85 percent of the number of units per tablet that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter using the procedure described in paragraph (e)(1) of that section.

§ 448.110b Bacitracin polymyxin tablets.

(a) *Requirements for certification*—(1) Bacitracin-polymyxin tablets conform to all requirements prescribed by § 448.110a(a) for bacitracin tablets, and are subject to all procedures prescribed by § 448.110a(a) for bacitracin tablets except that:

(i) Each tablets contains not less than 400 units of bacitracin.

(ii) Each tablet contains not less than 8,000 units of polymyxin B sulfate. The polymyxin B used conforms to the requirements prescribed for polymyxin by § 444.170a(a)(1) of this chapter.

(iii) [Reserved]

(iv) In addition to complying with the requirements of § 448.110a(a)(4) a person who requests certification of a batch shall submit with his request a statement showing the batch mark and (unless it was previously submitted) the results and the date of the latest tests and assays of the polymyxin B used in making the batch for potency and toxicity. He shall also submit in connection with his request (unless it was previously submitted) a sample consisting of 5 packages, each containing approximately equal portions of not less than 0.5 gram of the polymyxin B used in making the batch.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Bacitracin content.* Proceed as directed in § 448.110a(b)(1). Its content of bacitracin is satisfactory if it contains not less than 85 percent of the number of units it is represented to contain.

(ii) *Polymyxin content.* Using 10-percent potassium phosphate buffer, pH 6.0, prepare the sample as directed in paragraph (b)(1)(i) of this section, and proceed as directed in § 444.170a(b)(2) of this chapter. Its contents of polymyxin is satisfactory if it contains not less than 85 percent of the number of units that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 440.180a(b)(3) of this chapter.

§ 448.121 Colistin sulfate for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Colistin sulfate for oral suspension is a dry mixture of colistin sulfate, with or without one or more suitable and harmless buffer substances, suspending and dispersing agents, diluents, colorings, and flavorings. The colistin sulfate content is 5.0 milligrams of colistin per milliliter of the reconstituted suspension. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of colistin that it is represented to contain. The loss on drying is not more than 3.0 percent. The pH of the reconstituted suspension is not less than 5.0 and not more than 6.0. The colistin sulfate used conforms to the standards prescribed by § 448.21(a)(1).

(2) *Labeling.* It shall be labeled as prescribed in § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The colistin sulfate used in making the batch for potency, safety, loss on drying, pH, and identity.

(*b*) The batch for potency, loss on drying, and pH.

(ii) Samples required:

(*a*) The colistin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 6 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute the suspension as directed in the label. Remove an accurately measured representative portion of the reconstituted suspension with a hypodermic needle and syringe and dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 1.0 microgram of colistin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the suspension when reconstituted as directed in the labeling.

Subpart C—Injectable Dosage Forms

§ 448.210 Sterile bacitracin.

The requirements for certification and the tests and methods of assay for sterile bacitracin packaged for dispensing are described in § 448.10a.

§ 448.215 Sterile capreomycin sulfate.

The requirements for certification and the tests and methods of assay for sterile capreomycin sulfate packaged for dispensing are described in § 448.15a.

§ 448.220 Colistimethate sodium injectable dosage forms.

§ 448.220a Sterile colistimethate sodium.

The requirements for certification and the tests and methods of assay for sterile colistimethate sodium packaged for dispensing are described in § 448.20a.

§ 448.220b Colistimethate sodium for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Colistimethate sodium for injection in a dry mixture of colistimethate sodium and a suitable local anesthetic with or without a suitable buffer substance and preservative. Its content of colistimethate is satisfactory if it is not less than 90 percent nor more than 120 percent of the number of milligrams of colistin base equivalent that it is represented to contain. It is sterile. It is nonpyrogenic. It passes the safety test. When reconstituted as directed in the labeling, its pH is not less than 5.5 and not more than 6.5. Its loss on drying is not more than 9 percent. The colistimethate sodium used conforms to the standards prescribed by § 448.20a(a)(1), (i), (v), (vi), (vii), (viii), and (ix).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The colistimethate sodium used in making the batch for potency, histamine,

pH, loss on drying, heavy metals, and identity.

(b) The batch for potency, sterility, safety, pyrogens, loss on drying, and pH.

(ii) Samples required:

(a) The colistimethate sodium used in making the batch: 10 containers, each containing approximately 500 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 12 vials or, if each vial contains less than 150 milligrams of colistimethate, a minimum of 25 vials.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if the container is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute an aliquot with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 1.0 microgram of colistin base equivalent per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 5 milligrams of colistimethate per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this chapter.

(5) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using the solution prepared as directed in the labeling.

§ 448.280 Sterile viomycin sulfate.

The requirements for certification and the tests and methods of assay for sterile viomycin sulfate packaged for dispensing are described in § 448.80a.

Subpart D—Ophthalmic Dosage Forms

§ 448.310 Bacitracin ophthalmic dosage forms.

§ 448.310a Bacitracin ophthalmic.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Bacitracin ophthalmic is bacitracin, with or without one or more suitable and harmless preservatives, local anesthetics, buffer substances, and diluents. It is sterile. Its moisture content is not more than 5 percent. The bacitracin is of such quantity that when dissolved as directed in its labeling the potency of such solution is not less than 500 units per milliliter and maintains its labeled potency after it has been kept for 7 days at room temperature. Such solution has a pH of 6.5±0.5. The bacitracin used conforms to the requirements of § 448.10a(a)(1), except § 448.10a(a)(i), (ii), (iv), and (viii). Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* The immediate container of bacitracin ophthalmic shall be a tight container as defined by the U.S.P.; its closure shall be one through which a hypodermic needle cannot be introduced; and the immediate container and closure shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded. Each such container shall contain not less than 5,000 units, and each may be packaged in combination with a container of the solvent, sterile distilled water U.S.P.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the outside wrapper or container and the immediate container:

(a) The statement "Expiration date ----------", the blank being filled in with the date that is 12 months after the month during which the batch was certified.

(b) The statement "*Warning*—Not for injection".

(ii) If a local anesthetic is present, after the name "bacitracin ophthalmic", wherever it appears, the words "with ---------", (the blank being filled in with the established name of the local anesthetic) in juxtaposition with such name.

(4) *Requests for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless it was previously submitted) the date on which the latest assay of the bacitracin used in making such batch was completed, the number of units in each immediate container, the date on which the latest assay of the drug comprising such batch was completed, the quantity of each other ingredient used in making the batch, and a statement that each such ingredient used conforms to the requirements prescribed therefor by this section. If such batch or any part thereof is to be packaged with a solvent, such request shall also be accompanied by a statement that such solvent conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided in paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request results of the tests and assays listed after each of the following, made by him on an accurately representative sample of:

(a) The batch; potency, sterility, moisture, and pH of the solution prepared as directed in its labeling.

(b) The bacitracin used in making the batch; potency, toxicity, moisture, pH, and ash content.

(iii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request in the quantities hereinafter indicated accurately representative samples of the following:

(a) The batch:

(1) For all tests except sterility: One immediate container for each 5,000 immediate containers in the batch, but in no case less than five immediate containers.

Such samples shall be collected by taking single immediate containers at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(2) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(b) The bacitracin used in making the batch; 6 packages, each containing approximately equal portions of not less than 500 milligrams packaged in accordance with the requirements of § 448.10a(a)(2).

(c) In case of an initial request for certification, each other ingredient used in making the batch; one package of each, containing approximately 5.0 grams.

(iv) No result referred to in paragraph (a)(4)(ii)(b) of this section, and no sample referred to in paragraph (a)(4)(iii)(b) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 448.10a(b)(i).

(2) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) of this chapter.

(3) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using a solution prepared as directed in the labeling for the drug.

(4) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

§ 448.310b Sterile bacitracin-neomycin sulfate-polymyxin B sulfate ophthalmic ointment; sterile bacitracin-neomycin sulfate-polymyxin B sulfate-hydrocortisone acetate ophthalmic ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength quality, and purity.* Sterile bacitracin-neomycin sulfate-polymixin B sulfate ophthalmic ointment is composed of bacitracin, neomycin sulfate, and polymyxin B sulfate in a petrolatum base. Sterile bacitracin-neomycin sulfate-polymyxin B sulfate-hydrocortisone acetate ophthalmic ointment is composed of bacitracin, neomycin sulfate, polymyxin B sulfate, and hydrocortisone acetate in a petrolatum base. Each gram of ointment contains 500 units of baci-

tracin, 3.5 milligrams of neomycin, 10,000 units of polymyxin B, and if it is represented to contain the stearoid, either 5 milligrams or 10 milligrams of hydrocortisone acetate. They are sterile. Their moisture content is not more than 1 percent. The bacitracin used conforms to the standards prescribed therefor by § 448.10a(a)(1). The neomycin sulfate used conforms to the standards prescribed therefor by § 444.42a(a)(1) of this chapter. The polymyxin B sulfate used conforms to the standards prescribed therefor by § 448.30a(a)(1) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* It shall be packaged in collapsible tubes which shall be well-closed containers as defined by the U.S.P., and which shall not be larger than the ⅛-ounce size.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on the outside wrapper or container and the immediate container the statement "Expiration date _____", the blank being filled in with the date that is not more than 12 months after the month during which the batch was certified.

(4) *Requests for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch marks and (unless they were previously submitted) the date(s) on which the latest assays of the antibiotics used in making such batch were completed, the quantity of each ingredient used in making the batch, the date on which the latest assays of the drugs comprising such batch were completed, and that each component of the ointment base used conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request results of the tests and assays listed after each of the following, made by him on an accurately representative sample of:

(*a*) The batch: Bacitracin content, neomycin content, polymyxin B content, sterility, and moisture.

(*b*) The bacitracin used in making the batch: Potency, toxicity, pH, and ash content.

(*c*) The neomycin sulfate used in making the batch: Potency, toxicity, pH, and identity.

(*d*) The polymyxin B sulfate used in making the batch: Potency, toxicity, pH residue on ignition, and identity.

(iii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch:

(*1*) For all tests except sterility: One package for each 5,000 packages in the batch, but in no case less than seven packages, collected by taking single packages at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(2) For sterility testing: 20 packages collected at regular intervals throughout each filling operation.

(*b*) The bacitracin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*d*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*e*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 200 grams, except if a steroid is used, such package shall contain approximately 100 milligrams.

(iv) Neither the results referred to in paragraph (a)(4)(ii) (*b*), (*c*), and (*d*) of this section nor the samples referred to in paragraph (a)(4)(iii) (*b*), (*c*), and (*d*) of this section are required if such results or samples have been previously submitted.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Bacitracin content.* Proceed as directed in § 448.510c(b)(1)(i). Its content of bacitracin is satisfactory if it contains not less than 90 percent and not more than 140 percent of the number of units of bacitracin per gram that it is represented to contain.

(ii) *Polymyxin B content.* Proceed as directed in § 448.510c(b)(1)(ii), except calculate from the quantity of neomycin found (using the method prescribed in paragraph (b)(1)(iii) of this section) the quantity of neomycin that would be present when the sample is diluted to contain 10 units of polymyxin B (labeled potency) per milliliter. Prepare the polymyxin standard curve by adding this calculated quantity of neomycin to each concentration of polymyxin used for the curve. Use this standard curve to calculate the polymyxin content of the sample. Its content of polymyxin B is satisfactory if it contains not less than 90 percent and not more than 140 percent of the number of units of polymyxin B that it is represented to contain.

(iii) *Neomycin content.* Proceed as directed in § 448.510d(b)(1)(ii). Its content of neomycin is satisfactory if it contains not less than 90 percent and not more than 140 percent of the number of milligrams of neomycin that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(3) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 448.310c Bacitracin ointment; zinc bacitracin ointment.

The requirements for certification and the tests and methods of assay for bacitracin ointment and for zinc bacitracin ointment are described in § 448.510a.

§ 448.313 Sterile zinc bacitracin—neomycin sulfate—polymyxin B sulfate ophthalmic ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Zinc bacitracin—neomycin sulfate—polymyxin B sulfate ophthalmic ointment is zinc bacitracin, neomycin sulfate, and polymyxin B sulfate in a suitable and harmless ointment base. Each gram contains:

(i) 400 units of zinc bacitracin; 3.5 milligrams of neomycin, and 5,000 units of polymyxin B; or

(ii) 500 units of zinc bacitracin, 3.5 milligrams of neomycin, and 10,000 units of polymyxin B.

Its zinc bacitracin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of bacitracin that it is represented to contain. Its neomycin sulfate content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of neomycin that it is represented to contain. Its polymyxin B sulfate content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of polymyxin B that it is represented to contain. It is sterile. Its moisture content is not more than 0.5 percent. The zinc bacitracin used conforms to the standards prescribed by § 448.13(a)(1) of this chapter. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1) of this chapter. The polymyxin B sulfate used conforms to the standards prescribed by § 448.30a(a)(1) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The zinc bacitracin used in making the batch for potency, safety, loss on drying, pH, and zinc content.

(*b*) The neomycin sulfate used in making the batch for potency, safety, loss on drying, pH, and identity.

(*c*) The polymyxin B sulfate used in making the batch for potency, safety, loss on drying, pH, residue on ignition, and identity.

(*d*) The batch for zinc bacitracin content, neomycin content, polymyxin B content, sterility, and moisture.

(ii) Samples required:

(*a*) The zinc bacitracin used in making the batch: 10 packages, each containing 300 milligrams.

(*b*) The neomycin sulfate used in making the batch: 10 packages, each containing 300 milligrams.

(*c*) The polymyxin B sulfate used in making the batch: 10 packages, each containing 300 milligrams.

(*d*) The batch:

(*1*) For all tests except sterility: A minimum of 17 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Zinc bacitracin content.* Proceed as directed in § 436.105 of this chapter, except add to each standard response line concentration sufficient 0.01*N* hydrochloric acid to yield the same ratio of 0.01*N* hydrochloric acid to 1 percent potassium phosphate buffer, pH 6.0 (solution 1), as present in the sample solution diluted to the reference concentration. Prepare the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.01*N* hydrochloric acid and shake well. Allow the layers to separate. Remove the acid layer and repeat the extraction procedure with each of three more 20-to-25-milliliter quantities of 0.01*N* hydrochloric acid. Combine the acid extractives in a suitable volumetric flask and bring to mark with 0.01*N* hydrochloric acid. Remove an aliquot and further dilute with 1 percent potassium phospate buffer, pH 6.0 (solution 1), to the reference concentration of 1 unit of bacitracin per milliliter (estimated).

(ii) *Neomycin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of three more 20-to-25- milliliter quantities of solution 3. Combine the buffer extractives in a suitable volumetric flask and bring to mark with solution 3. Remove an aliquot and further dilute with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(iii) *Polymyxin B content.* Proceed as directed in § 436.105 of this chapter, except add to each concentration of the polymyxin standard response line a quantity of neomycin to yield the same concentration of neomycin as that present when the sample is diluted to contain 10 units of polymyxin per milliliter. Prepare the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 10 percent potassium phosphate buffer, pH 6.0 (solution 6), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of three more 20-to-25-milliliter quantities of solution 6. Combine the buffer extractives in a suitable volumetric flask and bring to mark with solution 6. Remove an aliquot and further dilute with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(3) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 448.321 Colistin sulfate for ophthalmic solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Colistin sulfate for ophthalmic solution is a dry mixture of colistin sulfate and mannitol packaged in combination with a suitable and harmless diluting solution which contains buffers and a preservative. When reconstituted as directed in the labeling, each milliliter contains 1.2 milligrams of colistin. Its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of colistin that it is represented to contain. It is sterile. Its loss on drying is not more than 5 percent. When reconstituted as directed in the labeling, its pH is not less than 5.5 and not more than 6.3. The colistin sulfate used conforms to the standards prescribed by § 448.21(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The colistin sulfate used in making the batch for potency, safety, loss on drying, pH, and identity.

(*b*) The batch for potency, sterility, loss on drying, and pH.

(ii) Samples required:

(*a*) The colistin sulfate used in making the batch: 10 containers, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 6 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Remove an accurately measured representative portion of the reconstituted solution and dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 1.0 microgram of colistin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the solution reconstituted as directed in the labeling.

Subpart E—Otic Dosage Forms

§ 448.410 Bacitracin-neomycin undecylenate otic drops.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Bacitracin-neomycin undecylenate otic drops is bacitracin and neomycin undecylenate in a vegetable oil vehicle, with a suitable and harmless suspending agent. It may contain a suitable local anesthetic and cortisone or a suitable derivative of cortisone. Each milliliter contains not less than 500 units of bacitracin and the equivalent of not less than 3.5 milligrams of neomycin. The moisture content is not more than 1 percent. The bacitracin used conforms to the standards prescribed therefor by § 448.10a(a)(1), except § 448.10a(a)(1) (ii), (iii), (iv), and (viii). The neomycin undecylenate used has a potency of not less than 300 micrograms of neomycin base activity per milligram (calculated on the anhydrous basis), an undecylenic acid content of not less than 600 micrograms per milligram, and a moisture content of not more than 5.0 percent. Its pH in a solution containing 50 milligrams per milliliter in 80 percent methyl alcohol is not less than 6.5 and not more than 7.0. It gives a positive infra-red identity test for neomycin undecylenate. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* In all cases the immediate container shall be glass, so closed as to be a tight container as defined by the U.S.P., and of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused that are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) The statement "Expiration date _____", the blank being filled in with the date that is 12 months after the month during which the batch was certified, except that the blank may be filled in with the date that is 18 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time such drug as prepared by him complies with the standards prescribed by paragraph (a)(1) of this section.

(ii) If it contains one or more of the active ingredients specified in paragraph (a)(1) of this section, after the name "bacitracin-neomycin undecylenate otic drops", wherever it appears, the words "with _____", in juxtaposition with such name, the blank being filled in with

the established name of each such ingredient used.

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch marks and (unless they were previously submitted) the dates on which the latest assays of the bacitracin and neomycin undecylenate used in making such batch were completed, the number of units of bacitracin and the number of milligrams of neomycin in each milliliter, the quantity of each ingredient used in making the batch, the date on which the latest assay of the batch was completed, and a statement that each ingredient used in making the batch conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request results of tests and assays listed after each of the following, made by him on an accurately representative sample of:

(*a*) The batch: The number of units of bacitracin and the number of milligrams of neomycin per milliliter, and moisture content.

(*b*) The bacitracin used in making the batch: Potency, moisture, pH, and ash content.

(*c*) The neomycin undecylenate used in making the batch: Neomycin content, undecylenic acid content, moisture, pH, and identity.

(iii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch: One immediate container for each 5,000 immediate containers in the batch, but in no case less than six immediate containers, collected by taking single immediate containers at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(*b*) The bacitracin used in making the batch: Six packages containing approximately equal portions of not less than 0.5 gram.

(*c*) The neomycin undecylenate used in making the batch: Ten packages, each containing approximately equal portions of not less than 300 milligrams.

(*d*) In case of an initial request for certification, each other substance used in making the batch: One package of each containing approximately 5 grams.

(iv) The results referred to in paragraph (a)(4)(ii) (*b*) and (*c*) of this section and the samples referred to in paragraph (a)(4)(iii) (*b*) and (*c*) of this section are not required if such results or samples have been previously submitted.

(b) *Tests and methods of assay*—(1) *Otic drops*—(i) *Potency*—(*a*) *Bacitracin content.* Proceed as directed in § 448.10a (b)(1) except § 448.10a(b)(1)(iii), and in lieu of the directions in § 448.10a(b)(1)(i)(*b*) prepare the sample as follows: Place an accurately measured representative portion of the sample (usually approximately 1 milliliter) in a blending jar containing 1.0 milliliter of polysorbate 80 and sufficient 1 percent phosphate buffer, pH, 6.0, to give a final volume of 200 milliliters. Using a high-speed blender, blend the mixture for 2 minutes and then make the proper dilutions in 1 percent phosphate buffer, pH 6.0, to one unit per milliliter (estimated). Its content of bacitracin is satisfactory if it contains not less than 85 percent of the number of units that it is represented to contain.

(*b*) *Neomycin content.* Proceed as directed in § 436.517(b)(1) of this chapter, except prepare the sample for assay as follows: Place an accurately measured representative portion of the sample (usually approximately 1 milliliter) in a 100-milliliter volumetric flask containing 5.0 milliliters chloroform, and mix well. Fill the flask to volume with 95 percent ethyl alcohol and mix well. Dilute an aliquot with 0.1*M* potassium phosphate buffer, pH 8.0, to either reference point, depending on the organism used. Its content of neomycin is satisfactory if it contains not less than 85 percent of the number of milligrams of activity that it is represented to contain.

(ii) *Moisture.* Proceed as directed in § 436.201(c) of this chapter.

(2) *Neomycin undecylenate used in making the otic drops*—(i) *Neomycin Content.* Proceed as directed in § 436.517(b) (1) of this chapter, except prepare the sample for assay as follows: Accurately weigh approximately 500 milligrams of neomycin undecylenate, transfer to a 250-milliliter volumetric flask, add 50 milliliters of chloroform, and mix well. Adjust to volume with 95 percent ethyl alcohol, and mix well. Dilute an aliquot with 0.1*M* potassium phosphate buffer, pH 8.0, to either reference point, depending on the organism used.

(ii) *Undecylenic acid content.* (*a*) Reagents:

95 percent ethyl alcohol (aldehyde-free).

Phenolphthalein indicator: Dissolve 1 gram of phenolphthalein in 100 milliliters of alcohol.

Alcoholic sodium hydroxide (about 0.1 normal): Weigh rapidly about 5 grams of sodium hydroxide pellets, dissolve in 10 milliliters of water, and add sufficient aldehyde-free alcohol to make 1 liter. Allow the solution to stand in a tightly stoppered bottle protected from light for 24 hours, then quickly decant the clear supernatant into a suitable tight container, and store protected from light. Determine the undecylenic acid equivalent of this solution each time an assay is performed.

(*b*) Procedure: Weigh accurately two 100-milligram portions of undecylenic acid (N.F. grade), and place each into separate Erlenmeyer flasks. Also, make two accurate weighings of the neomycin undecylenate sample, each consisting of about 120 milligrams, and place each into separate Erlenmeyer flasks. Carefully dissolve each of the undecylenic acid authentics and each of the samples in 10 milliliters of 95 percent ethyl alcohol. Add two drops of phenolphthalein indicator to each of the four flasks. Titrate each sample with 0.1*N* alcoholic sodium hydroxide to a phenolphthalein endpoint. Determine the amount of alcoholic sodium hydroxide equivalent to 10 milliliters of 95 percent ethyl alcohol and make this reagent-blank correction for each titration. From the undecylenic acid equivalent of the alcoholic sodium hydroxide, calculate the undecylenic acid content of neomycin undecylenate.

(iii) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) of this chapter.

(iv) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, except use a solution containing 50 milligrams per milliliter in 80 percent methyl alcohol.

(v) *Identity.* Weigh approximately 1 milligram of neomycin undecylenate and mix thoroughly with 200 milligrams of dried infrared spectrophotometric quality potassium bromide in a small ball mill for about 1 minute. Prepare a potassium bromide disk by transferring the uniform mixture to a dye of suitable size. Evacuate gradually while raising the pressure to 3,000 pounds per square inch. Hold at 3,000 pounds per square inch until evacuation is complete and then raise the pressure to 20,000 pounds per square inch. Hold the pressure for 2 or 3 minutes. Using a suitable infrared spectrophotometer, run the spectrum from 2 microns to 16 microns. Use an attenuator if necessary. Characteristic undecylenic acid peak absorbancies occur at 7.1 and 11 microns, distinguishing neomycin undecylenate from neomycin sulfate.

§ 448.421 Colistin sulfate-neomycin sulfate-thonzonium bromide-hydrocortisone acetate otic suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Colistin sulfate-neomycin sulfate-thonzonium bromide-hydrocortisone acetate otic suspension is a suspension containing colistin sulfate, neomycin sulfate, thonzonium bromide, and hydrocortisone acetate, and one or more preservatives, dispersing agents, and buffer substances. Each milliliter contains 3.0 milligrams of colistin, 3.3 milligrams of neomycin, 0.5 milligram of thonzonium bromide, and 10 milligrams of hydrocortisone acetate. Its content of colistin is satisfactory if it is not less than 90 percent and not more than 135 percent of the number of milligrams of colistin per milliliter that it is represented to contain. Its content of neomycin is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of neomycin per milliliter that it is represented to contain. It is sterile. Its pH is not less than 4.8 and not more than 5.2. The colistin sulfate used conforms to the standards prescribed therefor by § 448.21 (a)(1), except for safety. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1) (i), (v), (vi), and (vii) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the

requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The colistin sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The neomycin sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*c*) The batch for colistin content, neomycin content, sterility, and pH.

(ii) Samples required:

(*a*) The colistin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The batch:

(*1*) For all tests except sterility: A minimum of six immediate containers.

(*2*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Colistin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Thoroughly mix the sample and transfer an accurately measured representative portion of the sample into a 100-milliliter volumetric flask. Fill the flask to mark with 10 percent potassium phosphate buffer, pH 6.0 (solution 6). Further dilute with solution 6 to the reference concentration of 1.0 microgram of colistin per milliliter (estimated).

(ii) *Neomycin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Thoroughly mix the sample and transfer an accurately measured representative portion into a 100-milliliter volumetric flask. Fill the flask to mark with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3). Further dilute with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except transfer 0.25 milliliter of sample in lieu of 1 milliliter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted suspension.

§ 448.430 Polymyxin B sulfate otic solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Polymyxin B sulfate otic solution is polymyxin B sulfate with or without one or more suitable and harmless preservatives and buffer substances, in a suitable and harmless vehicle. Each milliliter contains 10,000 units of polymyxin B. It may contain 5.0 milligrams of hydrocortisone per milliliter. It is sterile. If it contains acetic acid, its pH is not less than 3.8 and not more than 4.3. The polymyxin B sulfate used conforms to the standards of § 448.30a(a)(1) (i), (v), (vi), (vii), and (ix). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The polymyxin B sulfate used in making the batch for potency, moisture, pH, residue on ignition, and identity.

(*b*) The batch for potency, sterility, and, if it contains acetic acid, pH.

(ii) Samples required:

(*a*) Polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 5 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each, containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 448.30a(b)(1), except prepare the sample by diluting volumetrically a representative portion (usually 1.0 milliliter) in 10 percent potassium phosphate buffer, pH 6.0, to give a stock solution of convenient concentration. Further dilute an aliquot in 10 percent potassium phosphate buffer, pH 6.0, to give a final estimated concentration of 10 units of polymyxin B per milliliter. Its content of polymyxin B is satisfactory if it contains not less than 90 percent and not more than 130 percent of the number of units of polymyxin B that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except if the steroid prevents solubilization, use 0.25 milliliter of the sample in lieu of 1 milliliter and proceed as directed in paragraph (e)(2) of that section.

(3) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using the undiluted solution.

Subpart F—Dermatologic Dosage Forms

§ 448.510 Bacitracin dermatologic dosage forms.

§ 448.510a Bacitracin ointment; zinc bacitracin ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Bacitracin ointment and zinc bacitracin ointment are composed of bacitracin or zinc bacitracin in a suitable and harmless ointment base, or they are a powder composed of bacitracin or zinc bacitracin and one or more suitable and harmless diluents, dispersing agents, and preservatives which upon the addition of the quantity of water recommended in its labeling, produces an ointment. It may contain a suitable local anesthetic (if it is not intended for ophthalmic use), cortisone, or a suitable derivative of cortisone, one or more suitable sulfonamides, one or more suitable proteolytic enzymes. Its potency is not less than 500 units per gram. If it is intended for ophthalmic use, it is sterile. Its moisture content is not more than 1 percent, except if it is a powder its moisture content is not more than 5 percent. The zinc bacitracin used conforms to the standards prescribed therefor by § 448.13(a)(1) except § 448.13(a)(1)(ii). The bacitracin used conforms to the standards prescribed therefor by § 448.10a(a)(1) except § 448.10a(a)(1)(ii), (iii), (iv), and (viii). The bacitracin used in making the bacitracin ophthalmic ointment conforms to the requirements of § 448.10a(a)(1) except § 448.10a(a)(1) (iv) and (viii). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* If it is bacitracin or zinc bacitracin in an ointment base it shall be packaged in collapsible tubes, which shall be well-closed containers as defined by the U.S.P., and which shall not be larger than the 2-ounce size, except if it is labeled solely for hospital use; but in no case shall such ointment be packaged in containers other than collapsible tubes if it is labeled for ophthalmic use, and such tubes shall not be larger than the ⅛-ounce size. If it is a powder, it shall be packaged in immediate containers of glass which meet the tests for tight containers as defined by the U.S.P. The composition of the immediate container and closure shall be such as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling*—(i) *It is packaged for ophthalmic use and it contains a sulfonamide, or cortisone, or a derivative of cortisone or a proteolytic enzyme.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its label or labeling, as hereinafter indicated, the following:

(*a*) On the outside wrapper or container and the immediate container:

(*1*) The statement "Expiration date ----------", the blank being filled in with the date that is not more than 36 months after the month during which the batch was certified, except if it is zinc bacitracin ointment or if it contains one or more proteolytic enzymes, the blank is filled in with the date that is 24 months after the month during which the batch was certified, except that the blank may be filled in with the date that is 48 months or 60 months, after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time such drug as prepared by

him complies with the standards prescribed by paragraph (a) (1) of this section.

(2) If it is a powder, the statement "After the addition of water, the ointment may be stored in the refrigerator for 1 week without significant loss of potency".

(b) If it contains in addition to bacitracin or zinc bacitracin, one or more other active ingredients specified in paragraph (a) (1) of this section, after the name "bacitracin ointment", wherever such name appears, the words "with ----------", in juxtaposition with such name, the blank being filled in with the established name of each such other ingredient.

(ii) *It is not packaged for ophthalmic use and it does not contain a sulfonamide, or cortisone, or a derivative of cortisone, or a proteolytic enzyme.* Its label and labeling shall comply with the requirements of paragraph (a) (3) (i) of this section, except that in lieu of the statement "Caution: Federal law prohibits dispensing without prescription", the labeling of each package shall bear information that the drug is for use only in the prevention of infections in minor cuts and abrasions and a statement that use of the drug should be discontinued and a physician consulted if signs of infection or irritation appear.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The bacitracin or zinc bacitracin used in making the batch for potency, moisture, and pH, and for ash content if it is bacitracin or for zinc content if it is zinc bacitracin, and for toxicity if the ointment is intended for ophthalmic use.

(b) The batch for potency and moisture and for sterility if it is intended for ophthalmic use.

(ii) Samples required:

(a) The bacitracin used in making the batch: Six packages, each containing approximately 500 milligrams, packaged in accordance with the requirements of 448.10a(a) (2).

(b) The zinc bacitracin used in making the batch: Five packages, each containing approximately 1 gram, packaged in accordance with the requirements of § 448.310a(a) (2).

(c) The batch:

(1) For all tests except sterility: A minimum of five immediate containers.

(2) For sterility testing: Twenty immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* If it is bacitracin, proceed as directed in § 448.10a(b) (1), except § 448.10a(b) (1) (iii), and in lieu of the directions in § 448.10a(b) (1) (i) (b), prepare the sample as directed in § 141a.8 (a) of this chapter. If it is zinc bacitracin, use 0.01*N* HCl in lieu of 1-percent phosphate buffer, pH 6.0, for the extraction or blending operation, and in lieu of the directions in § 448.10a(b) (1) (i) (a), prepare the standard as follows. Prepare the stock solution as directed in § 448.10a(b) (1) (i) (a), except that the dilutions for assay are prepared to contain the same ratio of 0.01*N* HCl to 1-percent phosphate buffer, pH 6.0, as the sample under test. Its content of bacitracin or zinc bacitracin is satisfactory if it contains not less than 85 percent of the number of units per gram that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Sterility.* If the ointment is intended for ophthalmic use, proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e) (3) of that section.

§ 448.510b Bacitracin-tyrothricin ointment.

(a) *Requirements for certification.* Bacitracin-tyrothricin ointment conforms to all requirements prescribed by § 448.510a(a) for bacitracin ointment, and is subject to all procedures prescribed by § 448.510a(a) for bacitracin ointment, except paragraph (a) (6) of that section, and except that:

(1) It contains not less than 0.5 milligram of tyrothricin per gram of ointment.

(2) [Reserved]

(3) In lieu of the samples required by § 448.510a(a) (4) (iii) (a), a minimum of 6 packages of the batch should be submitted.

(b) *Tests and methods of assay.* Proceed as directed in § 448.510a(b). Its content of bacitracin is satisfactory if it contains not less than 85 percent of the number of units per gram it is represented to contain.

§ 448.510c Bacitracin-polymyxin ointment; zinc bacitracin-polymyxin ointment.

(a) *Requirements for certification.* Bacitracin-polymyxin ointment and zinc bacitracin-polymyxin ointment conform to all requirements and are subject to all procedures prescribed by § 448.510a(a) for bacitracin ointment and zinc bacitracin ointment, except that:

(1) Its content of bacitracin or zinc bacitracin is not less than 200 units per gram.

(2) It contains not less than 4,000 units of polymyxin B per gram. The polymyxin B used conforms to the requirements prescribed for polymyxin B by § 444.170a(a) (1) of this chapter, except the standard for toxicity.

(3) Its moisture content is not more than 0.5 percent.

(4) If it is in liquid form, it shall be packaged in immediate containers of transparent glass which meet the test for tight containers as defined by the U.S.P.

(5) Its expiration date shall be the date that is 12 months after the month during which the batch was certified, except that the date that is 18 months, 24 months, 36 months, 48 months, or 60 months after the month during which the batch was certified may be used if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time such drug as prepared by him complies with the standards of identity, strength, quality, and purity prescribed for the drug.

(6) In addition to complying with the requirements of § 448.510a(a) (3), if it is in liquid form, each package shall bear on the outside wrapper or container and the immediate container, the statements "Shake well" and "Not for injection".

(7) In addition to complying with the requirements of § 448.510a(a) (4), a person who requests certification of a batch shall submit with his request a statement showing the batch mark and (unless it was previously submitted) the results and date of the latest tests and assays of the polymyxin used in making the batch for potency. He shall also submit in connection with his request a sample consisting of not less than 6 packages of ointment and (unless it was previously submitted) a sample consisting of 5 packages containing equal portions of not less than 0.5 gram each of the polymyxin used in making the batch.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Bacitracin or zinc bacitracin content.* Proceed as directed in § 448.510a(b) (1). Its content of bacitracin or zinc bacitracin is satisfactory if it contains not less than 85 percent of the number of units per gram that it is represented to contain.

(ii) *Polymyxin content.* Proceed as directed in § 444.170a(b) (2) (i) of this chapter, except in lieu of the directions in § 444.170a(b) (2) (i) (g) for the preparation of the sample, prepare the sample by one of the following methods: Accurately weigh approximately 5 grams and transfer to a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake with four 25-milliliter portions of 10-percent potassium phosphate buffer (pH 6.0) and combine the extracts. However, if the ointment contains a water-soluble base, accurately weigh approximately 5 grams and place in a blending jar containing 1.0 milliliter of polysorbate 80 and sufficient 10 percent potassium phosphate buffer, pH 6.0, to give a final volume of 200 milliliters. Using a high-speed blender, blend the mixture for 2 minutes and then make the proper estimated dilutions using 10 percent phosphate buffer, pH 6.0. Its content of polymyxin is satisfactory if it contains not less than 85 percent of the number of units per gram that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Sterility.* If the ointment is intended for ophthalmic use, proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e) (3) of that section.

§ 448.510d Bacitracin-neomycin topical ointment; zinc bacitracin-neomycin topical ointment.

(a) *Requirements for certification.* Bacitracin-neomycin topical ointment and zinc bacitracin-neomycin topical ointment conform to all requirements and are subject to all procedures pre-

scribed by § 448.510a(a) for bacitracin ointment and zinc bacitracin ointment, except paragraph (a) (6) of that section and except that:

(1) It contains not less than 3.50 milligrams of neomycin per gram. The neomycin used conforms to the standards prescribed by § 444.42a(a)(1) (i), (v), and (vi) of this chapter.

(2) Its expiration date shall be the date that is 12 months after the month during which the batch was certified, except that if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for 18 months, 24 months, 36 months, 48 months, or 60 months at room temperature such drug as prepared by him complies with the standards of identity, strength, quality, and purity prescribed for the drug, the expiration date blank shall be filled in with the date that is 18 months, 24 months, 36 months, 48 months, or 60 months after the month during which the batch was certified.

(3) In addition to complying with the requirements of § 448.510a(a)(4), a per- shall submit with his request a statement showing the number of units of bacitracin or zinc bacitracin and the number of milligrams of neomycin in each gram of ointment, the batch mark, and (unless it was previously submitted) the results and the date of the latest tests and assays of the neomycin used in making the batch for potency, moisture, and pH. He shall also submit in connection with his request a sample consisting of not less than 6 packages of the ointment and (unless it was previously submitted) a sample consisting of 5 packages containing approximately equal portions of not less than 0.5 gram each of the neomycin used in making such batch.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Bacitracin or zinc bacitracin content.* Proceed as directed in § 448.510a(b)(1). Its content of bacitracin or zinc bacitracin is satisfactory if it contains not less than 85 percent of the number of units per gram that it is represented to contain.

(ii) *Neomycin content.* Prepare the sample as directed in § 141a.8(a) of this chapter, except in lieu of potassium phosphate buffer use 0.10*M* phosphate buffer (pH 7.8–8.0) and proceed as directed in § 436.517(b)(1). Its content of neomycin is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 448.510e Bacitracin - polymyxin - neomycin ointment.

(a) *Requirements for certification.* Bacitracin - polymyxin - neomycin ointment conforms to all requirements prescribed by § 448.510c(a) for bacitracin-polymyxin ointment, and is subject to all procedures prescribed by § 448.510c (a) for bacitracin-polymyxin ointment, except that:

(1) Its content of neomycin is not less than 3.0 milligrams per gram. The neomycin used conforms to the standards prescribed by § 444.42a(a)(1) (i), (v), and (vi) of this chapter.

(2) It may contain one or more suitable and harmless chemical antimicropial agents.

(3) In addition to the labeling prescribed by § 448.510c(a)(1)(v), each package shall bear on the outside wrapper or container and the immediate container the number of milligrams of neomycin in each gram of the batch.

(4) In addition to complying with the requirements of § 448.510c(a)(1)(vii), a person who requests certification of a batch of bacitracin-polymyxin-neomycin ointment shall submit with his request a statement showing the batch mark and (unless they were previously submitted) the results and date of the latest tests and assays of the neomycin used in making the batch for potency, moisture, and pH. He shall also submit in connection with his request a sample consisting of not less than 7 packages of bacitracin-polymyxin-neomycin ointment and (unless it was previously submitted) a sample consisting of 5 packages of the neomycin used in making the batch, each containing approximately 0.5 gram.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Bacitracin content.* Proceed as directed in § 448.510c(b)(1)(i). Its content of bacitracin is satisfactory if it contains not less than 85 percent of the number of units per gram that it is represented to contain.

(ii) *Polymyxin content.* Proceed as directed in § 448.510c(b)(1)(ii), except calculate from the quantity of neomycin found (using the method prescribed in paragraph (b)(1)(iii) of this section the quantity of neomycin that would be present when the sample is diluted to contain son requesting certification of a batch 10 units of polymyxin (labeled potency) per milliliter. Prepare the polymyxin standard curve by adding this calculated quantity of neomycin to each concentration of polymyxin used for the curve. Use this standard curve to calculate the polymyxin content of the sample. Its content of polymyxin is satisfactory if it contains not less than 85 percent of the number of units that it is represented to contain.

(iii) *Neomycin content.* Proceed as directed in § 448.510d(b)(1)(ii). Its content of neomycin is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Sterility.* If the ointment is intended for ophthalmic use, proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e) (3) of that section.

§ 448.510f Bacitracin - neomycin - polymyxin powder topical; zinc bacitracin-neomycin-polymyxin powder topical.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Bacitracin-neomycin-polymyxin powder topical and zinc bacitracin-neomycin-polymyxin powder topical are bacitracin or zinc bacitracin, neomycin, and polymyxin B, with or without suitable and harmless diluents, preservatives, and lubricants, and with or without hyaluronidase, chymotrypsin, and polyvinylpyrrolidone. Unless it is packaged with one or more suitable and harmless inert gases, each gram contains not less than 200 units of bacitracin or zinc bacitracin, not less than 1,600 units of polmyxin B, and not less than 3.5 milligrams of neomycin. It contains not more than an average of 10 microorganisms per gram. Its moisture content is not more than 7.0 percent, unless it is packaged with one or more suitable and harmless inert gases, in which case its moisture content is not more than 0.5 percent. The bacitracin used conforms to the standards prescribed therefor by § 448.10a(a)(1), except § 448.10a(a)(1) (ii) and (iv). The zinc bacitracin used conforms to the standards prescribed by § 448.13(a)(1). The neomycin used conforms to the standards prescribed by § 444.42a(a)(1) (i), (iv), (v), and (vi) of this chapter. The polymyxin B used conforms to the standards prescribed therefor by § 444.170a(a)(1) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* In all cases the immediate container shall be a tight container as defined by the U. S. P. The composition of the immediate container shall be such as will not cause any change in the strength, quality, or purity of the contents beyond any limits therefor in applicable standards, except that minor changes so caused that are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded. Each such container may contain one or more suitable and harmless inert gases; in which case it shall contain not less than 8,000 units of zinc bacitracin, not less than 70 milligrams of neomycin, and not less than 100,000 units of polymyxin B.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106 (b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the outside wrapper or container and the immediate container:

(*a*) The statement "Expiration date ----------", the blank being filled in with the date that is 12 months after the month during which the batch was certified, except that the blank may be filled in with the date that is 18 months, or 24 months after the month during which the batch was certified if the person who rquests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time such drug as prepared by him complies with the standards prescribed by paragraph (a)(1) of this section,

(*b*) The statement "Not sterile".

(ii) If it contains, in addition to the antibiotic drug, one or more other active ingredients specified in paragraph (a)(1) of this section, after the name "bacitracin-neomycin-polymyxin powder topical" or "zinc bacitracin-neomycin-polymyxin powder topical", wherever such name appears, the words "with _____", in juxtaposition with such name, the blank being filled in with the established name of each such other ingredient.

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless they were previously submitted) the dates on which the latest assays of the bacitracin or zinc bacitracin, neomycin, and polymyxin used in making such batch were completed, the number of units of bacitracin or zinc bacitracin and polymyxin B, and the number of milligrams of neomycin in each immediate container, the date on which the latest assays of the drugs comprising such batch were completed, the quantity of each ingredient used in making the batch, and a statement that each such ingredient conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided in paragraph (a)(4)(iv) of this section such person shall submit in connection with his request results of the tests and assays listed after each of the following made by him on an accurately representative sample of:

(*a*) The batch: The number of units of bacitracin or zinc bacitracin, the number of units of polymyxin, and the number of milligrams of neomycin, a microorganism count, and moisture.

(*b*) The bacitracin or zinc bacitracin used in making the batch: Potency, toxicity, moisture, pH, and zinc content if it is zinc bacitracin.

(*c*) The neomycin used in making the batch: Potency, toxicity, moisture, and pH.

(*d*) The polymyxin B used in making the batch: Potency and toxicity.

(iii) Except as otherwise provided by paragraph (a)(4)(iv) of this section such person shall submit in connection with his request, in the quantities hereinafter indicated accurately representative samples of the following:

(*a*) The batch: For all tests except microorganism count, one immediate container for each 5,000 immediate containers in the batch, but in no case less than seven immediate containers, collected by taking single immediate containers at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(*b*) For microorganism testing: Five immediate containers.

(*c*) The bacitracin or zinc bacitracin used in making the batch: Six packages, containing approximately equal portions of not less than 0.5 gram.

(*d*) The neomycin used in making the batch: Five packages containing approximately equal portions of not less than 0.5 gram.

(*e*) The polymyxin B used in making the batch: Five packages containing approximately equal portions of not less than 0.5 gram.

(*f*) In case of an initial request for certification, each other substance used in making the batch: One package of each containing approximately 5 grams.

(iv) The results referred to in paragraph (a)(4)(ii) (*b*), (*c*), and (*d*) of this section and the samples referred to in paragraph (a)(4)(iii) (*c*), (*d*), (*e*), and (*f*) of this section are not required if such results or samples have been previously submitted.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Dry powder*—(*a*) *Bacitracin content.* Proceed as directed in § 448.10a(b)(1). Its content of bacitracin is satisfactory if it contains not less than 85 percent of the number of units that it is represented to contain.

(*b*) *Zinc bacitracin content.* Proceed as directed in § 448.13(b)(1). Its content of zinc bacitracin is satisfactory if it contains not less than 85 percent of the number of units that it is represented to contain.

(*c*) *Neomycin content.* Proceed as directed in § 436.517(b)(1) of this chapter. Its content of neomycin is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(*d*) *Polymyxin B content.* Proceed as directed in § 444.170a(b)(2)(1) of this chapter. Its content of polymyxin B is satisfactory if it contains not less than 85 percent of the number of units that it is represented to contain.

(ii) *Powder packaged with inert gases.* Spray, as directed in the labeling, the entire contents of each container to be tested into a separate 2-liter Erlenmeyer flask, held in a horizontal position. Add 500 milliliters of 0.01*N* HCl and shake to dissolve the contents. Immediately remove aliquots of this solution and using the appropriate buffer solutions to make further dilutions, proceed as directed in paragraph (b)(1)(i) of this section Calculate the average total amount of each antibiotic expelled from the containers. The total potency is satisfactory if it contains not less than 85 percent of the number of units of zinc bacitracin and polymyxin and not less than 85 percent of the number of milligrams of neomycin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Microorganism count*—(i) *Conduct of test for bacteria*—(*a*) *Dry powder.* Using approximately 200 milligrams of powder from each of five separate immediate containers, proceed as directed in § 436.20(e)(1) of this chapter, except after the three washings transfer the entire filter membrane to the surface of medium G as described in § 436.20(b)(7) of this chapter. Incubate the plate for 5 days at 30° C. to 32° C. Count the number of colonies appearing on the filter pad and calculate therefrom the number of viable microorganisms per gram of powder.

(*b*) *Powder packaged with inert gases.* Thoroughly cleanse the valve of each container to be tested with a suitable disinfectant. Into an empty, sterile Erlenmeyer flask, stoppered with a cotton plug, spray about one-half of the contents of each of five separate immediate containers by removing the cotton plug temporarily and using aseptic technique. Allow the propellant to evaporate. To the dry residue, which should not exceed 1 gram, add 500 milliliters of diluting fluid C as described in § 436.20(c)(3) of this chapter, and swirl the flask to dissolve the contents. Then proceed as described in paragraph (b)(3)(i)(*a*) of this section.

(ii) *Conduct of test for molds and yeasts*—(*a*) *Dry powder.* Proceed as directed in § 436.20(e)(1) of this chapter, using approximately 200 milligrams from each of the five containers tested, except use the agar medium H as described in § 436.20(b)(8) of this chapter, and incubate at 22° C.—25° C. for 5 days. Count the number of colonies appearing on the filter pad and calculate therefrom the number of viable microorganisms per gram of powder.

(*b*) *Powder packaged with inert gases.* Proceed as directed in paragraph (b)(3)(i)(*b*) of this section, use agar medium H as described in § 436.20(b)(8) of this chapter, and incubate at 25° C. for 5 days.

(iii) *Evaluation of results.* The microorganism count of the sample is satisfactory if the average number of viable microorganisms is not more than 10 per gram of powder, or per container if it is packaged with inert gases.

Subpart G—Vaginal Dosage Forms

§ 448.610 Bacitracin tablets; zinc bacitracin tablets; bacitracin suppositories; zinc bacitracin suppositories (if they are represented for vaginal use).

The requirements for certification and the tests and methods of assay for bacitracin tablets; zinc bacitracin tablets; bacitracin suppositories; zinc bacitracin suppositories (if they are represented for vaginal use) are described in § 448.110a.

Subparts H–I—[Reserved]

Subpart J—Certain Other Dosage Forms

§ 448.910 Bacitracin for prescription compounding.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Bacitracin for prescription compounding is a white to brown, neutral, water-soluble polypeptide intended for use in the extemporaneous compounding of prescriptions by practicing pharmacists. It is so purified and dried that:

(i) Its potency is not less than 40 units of bacitracin per milligram.

(ii) It passes the toxicity test.

(iii) Its moisture content is not more than 5.0 percent.

(iv) Its pH in an aqueous solution of 10,000 units per milliliter is not less than 5.5 and not more than 7.5.

(v) Its residue on ignition is not more than 3.0 percent.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass, and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. Each such container shall contain 5 million units of bacitracin.

(3) *Labeling.* Each package shall bear on its outside wrapper or container and on the immediate container the following:

(i) The statement "Caution: Federal law prohibits dispensing without prescription".

(ii) The statement "Not sterile".

(iii) The batch mark.

(iv) The number of units of bacitracin activity in each milligram and the number of grams of bacitracin in the immediate container.

(v) The statement "Expiration date ----------", the blank being filled in with the date that is 12 months after the month during which the batch was certified, unless the use of a longer dating period has been approved in accordance with the provisions of § 432.5(a)(3) of this chapter.

(vi) The statement "The potency of this drug cannot be assured for longer than 60 days after the container is first opened for compounding a prescription".

(vii) The statements, "For use only in extemporaneous prescription compounding. Not for manufacturing use".

(4) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, toxicity, moisture, pH, and residue on ignition.

(ii) Samples required: A 0.5-gram portion for each 5,000 packages in the batch, but in no case less than 10 such portions. Each such portion shall be collected at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 448.10a(b)(1).

(2) *Toxicity.* Proceed as directed in § 448.10a(b)(4).

(3) *Moisture.* Proceed as directed in § 440.80a(a)(5)(i) of this chapter.

(4) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using a solution containing 10,000 units of bacitracin per milliliter.

(5) *Residue on ignition.* Proceed as directed in § 448.10a(b)(7).

§ 448.913 Zinc bacitracin for prescription compounding.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Zinc bacitracin for prescription compounding is the zinc salt of a kind of bacitracin or a mixture of two or more such salts intended for use in the extemporaneous compounding of prescriptions by practicing pharmacists. It is so purified and dried that:

(i) Its potency is not less than 40 units of bacitracin per milligram.

(ii) It passes the toxicity test.

(iii) Its moisture content is not more than 5.0 percent.

(iv) Its pH in a saturated aqueous solution is not less than 6.0 and not more than 7.5.

(v) Its zinc content is not more than 10 percent by weight on a moisture-free basis.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. Each such container shall contain 5 million units of bacitracin.

(3) *Labeling.* Each package shall bear on its outside wrapper or container and on the immediate container the following:

(i) The statement "Caution: Federal law prohibits dispensing without prescription".

(ii) The statement "Not sterile".

(iii) The batch mark.

(iv) The number of units of bacitracin activity in each milligram of the zinc bacitracin, and the number of grams of zinc bacitracin in the immediate container.

(v) The statement. "Expiration date ----------", the blank being filled in with the date that is 12 months after the month during which the batch was certified, unless use of a longer dating period has been approved in accordance with the provisions of § 432.5(a)(3) of this chapter.

(vi) The statement, "The potency of this drug cannot be assured for longer than 60 days after the container is first opened for compounding a prescription".

(vii) The statements, "For use only in extemporaneous prescription compounding. Not for manufacturing use".

(4) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, toxicity, moisture, pH, and zinc content.

(ii) Samples required: A 0.5-gram portion for each 5,000 packages in the batch, but in no case less than 10 such portions. Each such portion shall be collected at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 448.13(b)(1).

(2) *Toxicity.* Proceed as directed in § 448.13(b)(2).

(3) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) of this chapter.

(4) *pH.* Prepare a saturated aqueous solution, using approximately 100 milligrams of the sample per milliliter, and proceed as directed in § 440.80a(b)(5)(ii) of this chapter.

(5) *Zinc content.* Proceed as directed in § 448.13(b)(5).

§ 448.930 Polymyxin B sulfate in certain other dosage forms.

§ 448.930a Polymyxin B sulfate for prescription compounding.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Polymyxin B sulfate for prescription compounding is the sulfate salt of a kind of polymyxin or a mixture of two or more such salts intended for use in the extemporaneous compounding of prescriptions by practicing pharmacists. It is a white to buff-colored powder. It is so purified and dried that:

(i) Its potency is not less than 6,000 units of polymyxin B per milligram, on an anhydrous basis.

(ii) It passes the toxicity test.

(iii) Its moisture content is not more than 7.0 percent.

(iv) Its pH in an aqueous solution containing 5 milligrams per milliliter is not less than 5.0 and not more than 7.5.

(v) Its residue on ignition is not more than 5 percent.

(vi) It gives positive color identity tests for polymyxin.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. Each such container shall contain 100 million units of polymyxin B.

(3) *Labeling.* Each package shall bear on its outside wrapper or container and on the immediate container the following:

(i) The statement "Caution: Federal law prohibits dispensing without prescription".

(ii) The statement "Not sterile".

(iii) The batch mark.

(iv) The number of units of polymyxin B activity in each milligram of the polymyxin B sulfate and the number of grams of polymyxin B sulfate in the immediate container.

(v) The statement "Expiration date ----------", the blank being filled in with the date that is 12 months after the month during which the batch was certified, unless the use of a longer dating period has been approved in accordance with the provisions of § 432.5(a)(3) of this chapter.

(vi) The statement, "The potency of this drug cannot be assured for longer than 60 days after the container is first opened for compounding a prescription".

(vii) The statements, "For use only in extemporaneous prescription compounding. Not for manufacturing use".

(4) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, toxicity, moisture, pH, residue on ignition, and identity.

(ii) Samples required: A 0.5-gram portion for each 5,000 packages in the batch, but in no case less than 10 such portions. Each such portion shall be collected at such intervals throughout the entire time of packaging the batch

that the quantities packaged during the intervals are approximately equal.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 448.30a (b) (1).

(2) *Toxicity.* Proceed as directed in § 440.80a(b) (4) of this chapter, using a test dose of 0.5 milliliter of a solution containing 1,200 units per milliliter of sterilized U.S.P. saline T.S.

(3) *Moisture.* Proceed as directed in § 440.80a(b) (5) (i) of this chapter.

(4) *pH.* Proceed as directed in § 440.80a(b) (5) (ii) of this chapter, except use a solution containing 5 milligrams per milliliter.

(5) *Residue on ignition.* Proceed as directed in § 448.10a(b) (7) of this chapter.

(6) *Identity.* Proceed as directed in § 448.30a(b) (9).

§ 448.930b Sterile polymyxin B sulfate-benzalkonium chloride urethral lubricant.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile polymyxin B sulfate-benzalkonium chloride urethral lubricant is polymyxin B sulfate and benzalkonium chloride, with one or more suitable and harmless suspending agents, in a suitable and harmless base. It contains, in each gram, 5,000 units of polymyxin B and 330 micrograms of benzalkonium chloride. Its content of polymyxin B is satisfactory if it contains not less than 90 percent and not more than 130 percent of the number of units of polymyxin B that it is represented to contain. It is sterile. Its pH is not less than 4.0 and not more than 5.5. The polymyxin B sulfate used conforms to § 448.30a(a) (1) (i), (iv), (vi), (vii), and (ix). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The polymyxin B sulfate used in making the batch for potency, toxicity, pH, residue on ignition, and identity.

(*b*) The batch for potency, sterility, and pH.

(ii) Samples required.

(*a*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch.

(*1*) For all tests except sterility: A minimum of five immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay.*

(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay by either of the following methods:

(i) Accurately weigh a representative portion of the ointment (usually 1.0 gram) and transfer it to a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake with four 25 milliliter portions of 10 percent potassium phosphate buffer, pH 6.0 (solution 6), and combine the aqueous extractives. After adjusting the volume of the combined extractives to 100 milliliters with solution 6, remove an aliquot and further dilute with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(ii) Place an accurately weighed representative portion of the sample into a high-speed glass blender jar containing sufficient 1.0 milliliter polysorbate 80 and 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to obtain a stock solution of convenient concentration. Blend for 3 to 5 minutes. Further dilute an aliquot with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20(e) (1) of this chapter, except dissolve the ointment as follows: Aseptically transfer a portion of 0.25 gram from each of 10 immediate containers of the drug to 400 milliliters of diluting fluid D in an Erlenmeyer flask. Repeat the procedure on another 10 immediate containers. Swirl the flasks to dissolve the ointment.

(3) *pH.* Proceed as directed in § 440.80a (b) (5) (ii) of this chapter, using the undiluted sample.

PART 449—ANTIFUNGAL ANTIBIOTICS

Subpart A—Bulk Drugs

449.4 Amphotericin B.
449.4a Amphotericin B for us in parenteral products.
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449.150 Nystatin oral dosage forms.
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449.204 Amphotericin B for injection.

Subpart D—[Reserved]

Subpart E—Otic Dosage Forms

449.450 Nystatin-neomycin sulfate-polymyxin B sulfate-fludrocortisone acetate for otic solution.

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449.504c Amphotericin B lotion.
449.550 Nystatin dermatologic dosage forms.
449.550a Nystatin ointment.
449.550b Nystatin - iodochlorhydroxyquin ointment.
449.550c Nystatin-neomycin sulfate-gramicidin-triamcinolone acetonide ointment; nystatin-neomycin sulfate - gramicidin-fludrocortisone acetate ointment.
449.550d Nystatin cream.
449.550e Nystatin-neomycin sulfate-gramicidin-triamcinolone acetonide cream.
449.550f Nystatin topical powder.
449.550g Nystatin-neomycin sulfate-gramicidin topical powder.

Subpart G—Vaginal Dosage Forms

449.610 Candicidin vaginal dosage forms.
449.610a Candicidin vaginal ointment.
449.610b Candicidin vaginal tablets.
449.610c Candicidin vaginal capsules.
449.650 Nystatin vaginal tablets.

AUTHORITY: Sec. 507, 59 Stat. 463, as amended (21 U.S.C. 357).

Subpart A—Bulk Drugs

§ 449.4 Amphotericin B.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Amphotericin B is a yellow to golden-orange powder. It is insoluble in water at pH. 6.0 to 7.0, anhydrous alcohols, esters, ethers, benzene, and toluene. It is soluble in dimethylformamide and dimethylsulfoxide. It is so purified and dried that:

(i) Its potency is not less than 750 micrograms of amphotericin B per milligram on an anhydrous basis.

(ii) It contains not more than 15 percent of amphotericin A.

(iii) It passes the safety test.

(iv) Its loss on drying is not more than 5.0 percent.

(v) Its pH in a 3 percent aqueous suspension is not less than 6.0 and not more than 8.0.

(vi) It contains not more than 3.0 percent residue on ignition.

(vii) It passes the identity test.

(2) *Labeling.* In addition to the labeling prescribed by § 432.5(b) of this chapter, each package shall bear on its label the statements "Store below 10° C." and "Protect from light and moisture".

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, amphotericin A content, safety, loss on drying, pH, residue on ignition, and identity.

(ii) Samples required on the batch: 10 packages, each containing not less than 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient dimethylsulfoxide to give a stock solution of convenient concentration. Further dilute an aliquot with dimethylsulfoxide to a concentration of 20 micrograms of amphotericin B per milliliter (estimated). Remove an aliquot; dilute with 0.2*M* potassium phosphate buffer, pH 10.5 (solution 10), to the reference concentration of 1.0 microgram of amphotericin B per milliliter (estimated).

(2) *Amphotericin A content*—(i) *Amphotericin A.* Dry approximately 20 milligrams of the amphotericin A working standard as described in § 436.200(a) of this chapter. Accurately weigh the dried working standard and quantitatively transfer into a 200-milliliter volumetric flask. Add exactly 40.0 milliliters of dimethylsulfoxide and dissolve. Make to mark with methyl alcohol and mix thoroughly. Pipette 4.0 milliliters of this solution into a 50-milliliter volumetric flask. Add methyl alcohol to mark and mix thoroughly.

(ii) *Amphotericin B.* Dry approximately 50 milligrams of the amphotericin B working standard as described in § 436.200(a) of this chapter. Accurately weigh the dried working standard and quantitatively transfer into a 50-milliliter volumetric flask. Add 10 milliliters of dimethylsulfoxide and dissolve. Make to mark with methyl alcohol and mix thoroughly. Pipette 4.0 milliliters of this solution into a 50-milliliter volumetric flask. Add methyl alcohol to mark and mix thoroughly.

The standard solution should be used for 1 day only.

(iii) *Sample.* Accurately weigh about 50 milligrams of the sample to be tested and quantitatively transfer into a 50-milliliter volumetric flask. Add 10 milliliters of dimethylsulfoxide and dissolve. Make to mark with methyl alcohol and mix thoroughly. Pipette 4.0 milliliters of this solution into a 50-milliliter volumetric flask. Add methyl alcohol to mark and mix thoroughly.

(iv) *Blank.* Pipette 10 milliliters of dimethylsulfoxide into a 50-milliliter volumetric flask. Make to mark with methyl alcohol and mix. Pipette 4.0 milliliters of this solution into a 50-milliliter volumetric flask. Make to mark with methyl alcohol and mix thoroughly.

(v) *Procedure.* Use a suitable ultraviolet spectrophotometer and 1-centimeter silica cells. Adjust the instrument to zero with the blank solution. Measure the absorbances of the solutions of standard A, standard B, and the sample at 304 nanometers and at 282 nanometers. Calculate the absorptivity of each standard at both wavelengths:

Percent amphotericin

$$A=\frac{[(B\times S_1)-(b\times S_2)]\times 625}{W_s\times[(B\times a)-(b\times A)]}$$

where:

A=Absorptivity of amphotericin A standard at 282 nanometers;
B=Absorptivity of amphotericin B standard at 282 nanometers;
a=Absorptivity of amphotericin A standard at 304 nanometers;
b=Absorptivity of amphotericin B standard at 304 nanometers;
S_1=Absorbance of sample at 282 nanometers;
S_2=Absorbance of sample at 304 nanometers;
W_s=Weight of sample in grams (on an anhydrous basis).

(3) *Safety.* Proceed as directed in § 436.33 of this chapter.

(4) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using a 3.0 percent aqueous suspension.

(6) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(7) *Identity.* Using the solutions prepared as described in paragraph (b)(2) (ii), (iii), and (iv) of this section, record the absorption spectrum from 320 to 240 nanometers. Then dilute these solutions (1+9) with methyl alcohol and record the absorption spectrum from 400 to 320 nanometers. The sample exhibits absorption peaks at identical wavelengths with that of the amphotericin B standard. Depending on the amphotericin A content of the sample, a peak may occur at 304 nanometers.

§ 449.4a Amphotericin B for use in parenteral products.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Amphotericin B is a yellow to golden-orange powder. It is insoluble in water at pH 6.0 to 7.0, anhydrous alcohols, esters, ethers, benzene, and toluene. It is soluble in dimethylformamide and dimethylsulfoxide. It is so purified and dried that:

(i) Its potency is not less than 750 micrograms of amphotericin B per milligram on an anhydrous basis.

(ii) It contains not more than 5 percent of amphotericin A.

(iii) It passes the safety test.

(iv) Its loss on drying is not more than 5.0 percent.

(v) Its pH in a 3 percent aqueous suspension is not less than 3.5 and not more than 6.0.

(vi) It contains not more than 0.5 percent residue on ignition.

(vii) It passes the identity test.

(2) *Labeling.* In addition to the labeling prescribed by § 432.5(b) of this chapter, each package shall bear on its label the statements "Store below 10° C." and "Protect from light and moisture".

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, amphotericin A content, safety, loss on drying, pH, residue on ignition, and identity.

(ii) Samples required on the batch: 10 packages, each containing not less than 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient dimethylsulfoxide to give a stock solution of convenient concentration. Further dilute with dimethylsulfoxide to give a concentration of 20 micrograms of amphotericin B per milliliter (estimated). Dilute an aliquot with 0.2*M* potassium phosphate buffer, pH 10.5 (solution 10), to the reference concentration of 1.0 microgram of amphotericin B per milliliter (estimated).

(2) *Amphotericin A content.* Proceed as directed in § 449.4(b)(2).

(3) *Safety.* Proceed as directed in § 436.33 of this chapter.

(4) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using a 3.0 percent aqueous suspension.

(6) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(7) *Identity.* Proceed as directed in § 449.4(b)(7).

§ 449.10 Candicidin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Candicidin is a brown to yellow powder. It is sparingly soluble in water; very slightly soluble in ethyl alcohol, butyl alcohol, and acetone. It is so purified and dried that:

(i) Its potency is not less than 1,000 micrograms of candicidin per milligram on an anhydrous basis.

(ii) Its loss on drying is not more than 4 percent.

(iii) Its pH is not less than 8.0 nor more than 10.0 in a 1 percent aqueous suspension.

(iv) Its ultraviolet absorption spectrum is characteristic of a conjugated heptaene and is qualitatively the same as that of the candicidin working standard.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, and identity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve a portion of the sample in sufficient dimethylsulfoxide to yield an estimated concentration of 1,000 micrograms of candicidin activity per milliliter. Further dilute an aliquot with sterile distilled water to the reference concentration of 0.06 microgram of candicidin activity per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using a 1 percent aqueous suspension.

(4) *Identity*—(i) *Preparation of aqueous alcohol solution.* Prepare an aqueous alcohol solution by mixing 53 volumes of ethyl alcohol and 47 volumes of water.

(ii) *Preparation of standard solution.* Grind a small portion of the candicidin working standard to a fine powder with a mortar and pestle. Accurately weigh an amount equivalent to 20,000 micrograms of candicidin activity and transfer it to a 100-milliliter volumetric flask. Add about 50 milliliters of the aqueous alcohol solution and shake to effect complete dissolution. Bring to volume with the aqueous alcohol solution and mix well. Transfer a 25-milliliter aliquot to a 100-milliliter volumetric flask and bring to volume with the aque-

ous alcohol solution. This solution contains 50 micrograms of candicidin activity per milliliter.

(iii) *Preparation of sample solution.* Proceed as directed in paragraph (b) (4) (ii) of this section.

(iv) *Procedure.* Using a suitable recording spectrophotometer, record the absorption spectra of the standard solution and the sample solution between the wavelengths of 330 and 410 nanometers with the aqueous alcohol solution as the reference solution. Compare the absorption spectra of the standard solution and the sample solution. They should exhibit absorption maxima and minima at the same wavelengths, which are nominally 342, 359, 378, and 397 nanometers for the maxima and 348, 366, and 390 nanometers for the minima.

§ 449.20 Griseofulvin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Griseofulvin is a microsize, white to pale-cream compound with the following chemical name: 7-chloro-2',4,6-trimethoxy-6'β-methylspiro[benzofuran-2(3*H*),1'-[2]cyclohexene]-3,4'-dione. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram.

(ii) It passes the safety test.

(iii) Its loss on drying is not more than 1.0 percent.

(iv) Its melting point, after drying, is not less than 217° C. and not more than 224° C.

(v) Its specific rotation in dimethylformamide at 25° C. is not less than +348° and not more than +364°.

(vi) Its ultraviolet absorption spectrum in methyl alcohol compares qualitatively with that of the griseofulvin reference standard.

(vii) Its residue on ignition is not more than 0.2 percent.

(viii) Its heavy metals content is not more than 25 parts per million.

(ix) Its specific surface area is not less than 1.3 and not more than 1.7 square meters per gram.

(x) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 (b) of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, loss on drying, melting point, specific rotation, identity, residue on ignition, heavy metals, specific surface area, and crystallinity.

(ii) Samples required: 10 packages, each containing not less than 1 gram.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Spectrophotometric assay*—(a) *Preparation of sample and standard solutions.* Accurately weigh approximately 50 milligrams each of the sample and griseofulvin working standard. Dissolve each in exactly 100 milliliters of methyl alcohol and mix well. (The standard solution can be kept under refrigeration and used for up to 1 month.) Transfer 2.0-milliliter aliquots of the sample and standard to separate 100-milliliter volumetric flasks. Bring to mark with methyl alcohol, stopper, and mix well.

(b) *Procedure.* Adjust a suitable ultraviolet spectrophotometer to 100 percent light transmission with methyl alcohol in the absorption cell. Determine the absorbance values for the sample and standard at the absorption peak at 292 nanometers. If a recording spectrophotometer is used, record the ultraviolet absorption spectrum from 240 to 320 nanometers. If a nonrecording spectrophotometer is used, determine the absorbance value at the absorption peak. (The exact position of the peak should be determined for the particular instrument used.)

(c) *Calculations.* Determine the griseofulvin content of the sample as follows:

where:

A_1 = Absorbance of sample at 292 nanometers.

A_2 = Absorbance of standard at 292 nanometers.

$$\text{Micrograms of griseofulvin per milligram} = \frac{A_1}{A_2} \times \frac{\text{Milligrams of standard}}{\text{Milligrams of sample}} \times \text{Potency of the standard in micrograms per milligram.}$$

(ii) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an appropriate portion of the griseofulvin sample in dimethylformamide to give a stock solution of convenient concentration. Further dilute the stock solution with dimethylformamide to give a concentration of 100 micrograms of griseofulvin per milliliter. Then further dilute an aliquot of this solution with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 5.0 micrograms of griseofulvin per milliliter (estimated).

(2) *Safety.* Proceed as directed in § 436.33 of this chapter.

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *Melting point.* Proceed as directed in § 436.209 of this chapter.

(5) *Specific rotation.* Accurately weigh approximately 250 milligrams of the sample in a 25-milliliter glass-stoppered volumetric flask and dissolve in about 15 milliliters of dimethylformamide. Bring to volume with dimethylformamide, stopper, and mix well. Proceed as directed in § 436.210 of this chapter, using a 2.0-decimeter polarimeter tube.

(6) *Identity.* Record the ultraviolet absorption spectrum of solutions of the sample and standard prepared as directed in paragraph (b) (1) (i) of this section from 240 to 320 nanometers. The curves shall be similar, and each shall have a maximum at 292±2 nanometers and a minimum at 269±2 nanometers.

(7) *Residue on ignition.* Proceed as directed in § 436.207 of this chapter.

(8) *Heavy metals.* Proceed as directed in § 436.208 of this chapter.

(9) *Specific surface area*—(i) *Procedure.* Determine the apparent particle size in microns by the air-permeation method, using a suitable subsieve sizer. Weigh 1.819 grams ±0.001 gram of the sample and transfer to the compression tube of the apparatus. Compact the sample with moderate pressure so that it has a uniform porosity. Pass compressed dry air through the sample and measure the air pressure with a water manometer. Observe the porosity and calculate the apparent particle size from the instrument equation or read it from a chart that has been calculated in accordance with the equation. Repeat the readings at successively higher degrees of compaction until the apparent particle size reaches a minimum. Calculate the observed specific surface area (SSA) in square meters per gram of sample, as follows:

$$\text{Observed SSA} = \frac{6}{\text{Minimum apparent particle size in microns} \times 1.455 \times F}$$

where F is a factor used to correct the apparent particle size to the true particle size:

Porosity reading	F
0.80	1.3771
0.76	1.4142
0.72	1.4573
0.68	1.5082
0.64	1.5690
0.60	1.6432
0.56	1.7353
0.52	1.8528
0.48	2.0076
0.44	2.2203
0.40	2.5298

(ii) *Standard.* Determine the observed specific surface area of the griseofulvin specific surface area standard by the method prescribed in paragraph (b) (9) (i) of this section, using the same instrument and the same air pressure setting.

(iii) *Calculations.* Calculate the corrected specific surface area of the sample as follows:

$$\text{SSA of sample} = \frac{\text{Observed SSA of sample} \times \text{assigned SSA of standard}}{\text{Observed SSA of standard}}.$$

(10) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

§ 449.50 Nystatin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin is the yellow to light-tan compound of a kind of nystatin or a mixture of two or more such compounds. It is very slightly soluble in water, moderately soluble in methyl alcohol, butyl alcohol, or propyl alcohol. It is so purified and dried that:

(i) Its potency is not less than 2,000 units of nystatin per milligram.

(ii) It passes the safety test.

(iii) Its loss on drying is not more than 5.0 percent.

(iv) Its pH in a 3 percent aqueous suspension is not less than 6.5 and not more than 8.0.

(v) It passes the identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, loss on drying, pH, and identity.

(ii) Samples required on the batch: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient dimethylformamide to give a nystatin concentration of 400 units per milliliter (estimated). Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(2) *Safety.* Proceed as directed in § 436.33 of this chapter.

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a 3 percent aqueous suspension of the drug.

(5) *Identity.* Weigh approximately 100 milligrams of the sample into a 200-milliliter, glass-stoppered, volumetric flask. Add 50 milliliters of absolute methyl alcohol and 10 milliliters of glacial acetic acid. When the sample has dissolved, dilute to volume with methyl alcohol. Transfer 2 milliliters of this solution to a 100-milliliter volumetric flask and dilute to volume with methyl alcohol. Use the same dilution of acetic acid in methyl alcohol as the blank. Immediately determine the absorption peaks at 230, 291, 305, and 319 nanometers, and the shoulders at 279±2 nanometers, using a suitable ultraviolet spectrophotometer and quartz cells. Set the instrument to 100 percent transmission with the blank. If a recording spectrophotometer is used, record the ultraviolet absorption spectrum from 220 nanometers to 350 nanometers. If a nonrecording spectrophotometer is used, the exact positions of the peaks and shoulder should be determined for the particular instrument used. The ratio of the two absorbances

$$\left(\frac{A_{305}}{A_{291}}\right)$$

should be not less than 0.90 and not more than 1.25.

Subpart B—Oral Dosage Forms

§ 449.104 Amphotericin B oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Amphotericin B oral suspension is a mixture of amphotericin B with one or more suitable and harmless preservatives, colorings, sweetening ingredients, flavorings, buffer substances, lubricants, suspending agents, and sequestrants in an aqueous vehicle. Each milliliter contains 100 milligrams of amphotericin B. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of amphotericin B that it is represented to contain. Its pH is not less than 4.5 and not more than 6.0. The amphotericin B conforms to the standards prescribed by § 449.4(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The amphotericin B used in making the batch for potency, amphotericin A content, safety, loss on drying, pH, residue on ignition, and identity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The amphotericin B used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately measured representative portion into a high-speed glass blender with sufficient dimethylsulfoxide to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Dilute an aliquot of the stock solution with dimethylsulfoxide to give a concentration of 20 micrograms of amphotericin B per milliliter (estimated). Further dilute an aliquot with 0.2*M* potassium phosphate buffer, pH 10.5 (solution 10), to the reference concentration of 1.0 microgram of amphotericin B per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted suspension.

§ 449.120 Griseofulvin oral dosage forms.

§ 449.120a Griseofulvin tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Griseofulvin tablets are tablets composed of griseofulvin, with or without one or more suitable fillers, colorings, lubricants, and binders. Each tablet contains 125, 250, or 500 milligrams of griseofulvin. The potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of griseofulvin that it is represented to contain. The loss on drying is not more than 5.0 percent. They shall disintegrate within 1 hour. The griseofulvin used conforms to the standards prescribed by § 449.20(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The griseofulvin used in making the batch for potency, safety, loss on drying, melting point, specific rotation, identity, residue on ignition, heavy metals, specific surface area, and crystallinity.

(*b*) The batch for potency, loss on drying, and disintegration time.

(ii) Samples required:

(*a*) The griseofulvin used in making the batch: 10 packages, each containing not less than 1 gram.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Spectrophotometric assay.* Proceed as directed in § 449.20(b)(1)(i), except prepare the sample for assay as follows: Weigh accurately a representative number of tablets and determine the average weight per tablet. Reduce the tablets to a fine powder in a mortar and transfer an amount of powder containing 200 milligrams (estimate) of griseofulvin to a 200-milliliter glass-stoppered volumetric flask. Add about 100 milliliters of methyl alcohol to the flask. Warm the contents by heating the flask carefully over a steam bath. Swirl the contents several times during the heating period. Shake the flask on a mechanical shaker for 15 minutes, allow to cool to room temperature, and adjust to volume with methyl alcohol. Mix well. Filter 30 to 40 milliliters through paper, discarding the first 10 to 15 milliliters of filtrate. Transfer exactly 2.0 milliliters of the subsequent filtrate to a 200-milliliter volumetric flask, adjust to volume with methyl alcohol, and mix well. Using a suitable ultraviolet spectrophotometer and matched 1-centimeter quartz cells, set the instrument to 100 percent transmission with methyl alcohol. Determine the absorbance of the sample and working standard (prepared as described in § 449.20(b)(1)(i)(*a*) at the absorption peak at 292±2 nanometers. Calculate the milligrams of griseofulvin per tablet as follows:

$$\text{Milligrams of griseofulvin per tablet} = \frac{\text{Absorbance of sample} \times \text{average weight of tablet in milligrams} \times 200{,}000}{a_s \times \text{weight of powder tested in milligrams}}$$

where:

a_s = Absorptivity (1 percent, 1 cm.) of the griseofulvin working standard measured at 292±2 nanometers.

(ii) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Powder the tablets and weigh an amount of the powder as described in paragraph (a)(1)(i) of this section. Transfer the powder to a flask of 500-milliliter capacity. Add a sufficient quantity of dimethylformamide to give a stock solution of convenient concentration. Shake well and carefully warm the contents over a steam bath. Stopper the flask, place on an automatic shaking machine, and shake for 15 minutes. Transfer 25 to 30 milliliters to a centrifuge tube, close the tube, and centrifuge sufficiently to obtain a clear supernatant. Dilute an aliquot of the supernatant with dimethylformamide to give a concentration of 100 micrograms of griseofulvin per milliliter (estimated). Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 5.0 micrograms of griseofulvin per milliliter.

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter, using the procedure described in paragraph (e)(1) of that section.

§ 449.120b Griseofulvin capsules.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Griseofulvin capsules are gelatin capsules containing griseofulvin with a suitable filler and binder, with or without a suitable lubricant. Each capsule contains 125 or 250 milligrams of griseofulvin. The potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of griseofulvin that it is represented to contain. The loss on drying is not more than 1.0 percent. The griseofulvin used conforms to the standards prescribed by § 449.20(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The griseofulvin used in making the batch for potency, safety, loss on drying, melting point, specific rotation, identity, residue on ignition, heavy metals, specific surface area, and crystallinity.

(b) The batch for potency and loss on drying.

(ii) Samples required:

(a) The griseofulvin used in making the batch: 10 packages, each containing not less than 1 gram.

(b) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay—*(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Spectrophotometric assay.* Proceed as directed in § 449.20(b)(1)(i), except prepare the sample as follows: Weigh accurately the contents of a representative number of capsules and determine the average weight per capsule. Mix the contents of the capsules and transfer an amount of powder containing 200 milligrams (estimated) of griseofulvin to a 200-milliliter glass-stoppered volumetric flask. Add about 100 milliliters of methyl alcohol to the flask. Warm the contents by heating the flask carefully over a steam bath. Swirl the contents several times during the heating period. Shake the flask on a mechanical shaker for 15 minutes, allow to cool to room temperature, and adjust to volume with methyl alcohol. Mix well. Filter 30 to 40 milliliters through paper, discarding the first 10 to 15 milliliters of filtrate. Transfer exactly 2.0 milliliters of the subsequent filtrate to a 200-milliliter volumetric flask, adjust to volume with methyl alcohol, and mix well. Using a suitable ultraviolet spectrophotometer and matched 1-centimeter quartz cells, set the instrument to 100 percent light transmission with methyl alcohol. Determine the absorbance of the sample and working standard (prepared as described in § 449.20(b)(1)(i)(*a*)) at the absorption peak at 292±2 nanometers. Calculate the milligrams of griseofulvin per capsule as follows:

$$\text{Milligrams of griseofulvin per capsule} = \frac{\text{Absorbance of the sample} \times \text{average weight of capsule content in milligrams} \times 200{,}000}{a_s \times \text{weight of powder tested in milligrams}}$$

where:

a_s = Absorptivity (1 percent, 1 cm.) of the griseofulvin working standard measured at 292±2 nanometers.

(ii) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve the contents of a representative number of capsules in sufficient dimethylformamide to give a stock solution of convenient concentration. Dilute an aliquot of the stock solution with dimethylformamide to give a concentration of 100 micrograms of griseofulvin per milliliter (estimated). Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 5.0 micrograms of griseofulvin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

§ 449.120c Griseofulvin oral suspension.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Griseofulvin oral suspension is griseofulvin with one or more suitable flavorings, colorings, wetting agents, preservatives, and diluents in an aqueous vehicle. Each milliliter contains 25 milligrams of griseofulvin. Its potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of griseofulvin that it is represented to contain. Its pH is not less than 6.5 and not more than 7.5. The griseofulvin used conforms to the standards prescribed by § 449.20(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The griseofulvin used in making the batch for potency, safety, loss on drying, melting point, specific rotation, identity, residue on ignition, heavy metals, specific surface area, and crystallinity.

(b) The batch for potency and pH.

(ii) Samples required:

(a) The griseofulvin used in making the batch: 10 packages, each containing not less than 1 gram.

(b) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay—*(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Spectrophotometric assay.* Proceed as directed in § 449.20(b)(1)(i), except prepare the sample for assay as follows: Prepare a 0.50 milligram per milliliter solution (estimated) of the suspension by transferring an appropriate volume of the well-shaken suspension to a 100-milliliter volumetric flask. Add about 50 milliliters of methyl alcohol to the flask. Warm the contents by heating the flask carefully over a steam bath. Swirl the contents several times during the heating period. Shake the flask on a mechanical shaker for 15 minutes, allow to cool to room temperature, and adjust to volume with methyl alcohol. Mix well. Filter the solution, discarding the first 10 to 15 milliliters of filtrate. Transfer exactly 1.0 milliliter of the subsequent filtrate to a 50-milliliter volumetric flask, adjust to volume with methyl alcohol, and mix well. Using a suitable ultraviolet spectrophotometer and matched 1 centimeter quartz cells, set the instrument to 100 percent transmission with methyl alcohol. Determine the absorbance of the sample and working standard (prepared as described in § 449.20(b)(1)(i)(*a*)) at the absorption peak at 292±2 nanometers. Calculate the milligrams of griseofulvin per milliliter of sample as follows:

$$\text{Milligrams of griseofulvin per milliliter} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{Weight of standard in milligrams}}{\text{Milliliters of suspension tested}} \times \frac{\text{Potency of standard in micrograms per milligram}}{1{,}000}$$

(ii) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured volume of the well-shaken suspension with sufficient dimethylformamide to give a stock solution containing 100 micrograms of griseofulvin per milliliter (estimated). Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 5.0 micrograms of griseofulvin per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this subchapter, using the undiluted suspension.

§ 449.150 Nystatin oral dosage forms.

§ 449.150a Nystatin tablets.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Nystatin tablets are tablets composed of nystatin and suitable and harmless buffer substances, diluents, binders, lubricants, colorings, and flavorings. Each tablet contains 500,000 units of nystatin. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of nystatin that it is represented to contain. If they are plain coated, the loss on drying is not more than 5 percent, and they shall disintegrate within 2 hours. If they are film coated, and contain a starch filler, the loss on drying is not more than 8 percent, and they shall disintegrate within 30 minutes. The nystatin used conforms to the standards prescribed by § 449.50(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, safety, loss on drying, pH, and identity.

(*b*) The batch for potency, loss on drying, and disintegration time.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each consisting of not less than 300 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Blend a representative number of tablets for 3 to 5 minutes in a high-speed glass blender with sufficient dimethylformamide to give a convenient concentration. Dilute an aliquot with sufficient dimethylformamide to give a stock solution containing 400 units of nystatin per milliliter. Further dilute an aliquot with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter.

§ 449.150b Nystatin oral suspension.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Nystatin oral suspension is a suspension containing nystatin and one or more suitable preservatives, suspending agents, surfactants, flavorings, and colorings in purified water. Each milliliter contains 100,000 units of nystatin. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of nystatin that it is represented to contain. Its pH is not less than 4.5 and not more than 6.0; except, if the product contains glycerin, its pH is not less than 6.0 and not more than 7.5. The nystatin used conforms to the standards prescribed by § 449.50(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, safety, loss on drying, pH, and identity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately measured representative aliquot of the sample into a high-speed glass blender jar containing sufficient dimethylformamide to give a convenient concentration. Blend for 3 to 5 minutes. Dilute an aliquot with sufficient dimethylformamide to give a stock solution containing 400 units of nystatin per milliliter (estimated). Remove an aliquot of the stock solution and further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted suspension.

§ 449.150c Nystatin for oral suspension.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Nystatin for oral suspension is a dry powder consisting of nystatin, and suitable and harmless suspending substances, preservatives, diluents, colorings, and flavorings. When the suspension is prepared as directed in its labeling, each milliliter contains 100,000 units of nystatin. Its potency is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of nystatin that it is represented to contain. The pH of the reconstituted drug is not less than 4.9 and not more than 5.5. Its moisture content is not more than 7.0 percent. The nystatin used conforms to the standards prescribed by § 449.50(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, safety, loss on drying, pH, and identity.

(*b*) The batch for potency, moisture and pH.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each consisting of 300 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute the drug as directed in the labeling. Blend an appropriate aliquot in a high-speed glass blender for 3 to 5 minutes, using sufficient dimethylformamide to give a convenient concentration. Dilute an aliquot with sufficient dimethylformamide to give a stock solution containing 400 units of nystatin per milliliter. Further dilute an aliquot with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(2) *Moisture.* Using the dry powder, proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the suspension after reconstituting as directed in the labeling.

Subpart C—Injectable Dosage Forms

§ 449.204 Amphotericin B for injection.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Amphotericin B for injection is a dry powder containing in each immediate container 50 milligrams of amphotericin B, 41 milligrams of sodium desoxycholate, and suitable buffering substances. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of amphotericin B that it is represented to contain. It is sterile. It passes the safety test. It is nonpyrogenic. Its loss on drying is not more than 8.0 percent. Its pH in an aqueous solution containing 10 milligrams of amphotericin B per milliliter is not less than 7.2 and not more than 8.0. The amphotericin B used conforms to the standards prescribed by § 449.4a(a)(1).

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5 of this chapter, each package shall bear on its label and labeling the following statement: "For intravenous infusion in hospitals only".

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The amphotericin B used in making the batch for potency, amphotericin A content, loss on drying, pH, residue on ignition, and identity.

(b) The batch for potency, sterility, safety, pyrogens, loss on drying, and pH.

(ii) Samples required:

(a) Amphotericin B used in making the batch: 10 packages, each containing approximately equal portions of not less than 500 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 10 immediate containers.

(2) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Then using a suitable syringe and hypodermic needle, remove all of the withdrawable contents if the container is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with sufficient dimethylsulfoxide to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with dimethylsulfoxide to a concentration of 20 micrograms of amphotericin B per milliliter (estimated). Remove an aliquot of this solution and dilute with 0.2*M* potassium phosphate buffer, pH 10.5 (solution 10), to the reference concentration of 1.0 microgram of amphotericin B per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use 50 milligrams in lieu of 300 milligrams.

(3) *Safety.* Proceed as directed in § 436.33 of this chapter.

(4) *Pyrogens.* Proceed as directed in § 436.32(e) of this chapter, using a solution containing 2 milligrams of amphotericin B per milliliter, except in lieu of paragraph (a)(3), if no rabbit shows an individual rise in temperature of 1.1° C. or more above its respective control temperature, and if the sum of the three temperature rises does not exceed 3° C., the sample meets the requirements for absence of pyrogen. If one or two rabbits show a temperature rise of 1.1° C. or more, or if the sum of temperature rises exceeds 3° C., repeat the test using five other rabbits. If not more than three of the eight rabbits show a temperature rise of 1.1° C. or more, and if the sum of the temperature rises does not exceed 8° C. the sample meets the requirements for absence of pyrogens.

(5) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter using an aqueous solution containing 10 milligrams of amphotericin B per milliliter.

Subpart D—[Reserved]

Subpart E—Otic Dosage Forms

§ 449.450 Nystatin-neomycin sulfate-polymyxin B sulfate-fludrocortisone acetate for otic solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin-neomycin sulfate-polymyxin B sulfate-fludrocortisone acetate for otic solution is a dry mixture of nystatin, neomycin sulfate, polymyxin B sulfate, and fludrocortisone acetate. Its loss on drying is not more than 6.5 percent. It may be packaged in combination with an immediate container of a suitable and harmless aqueous diluent containing suitable preservatives and buffers. When reconstituted as directed in the labeling, each milliliter contains 100,000 units of nystatin, 3.5 milligrams of neomycin, 10,000 units of polymyxin B, and 1 milligram of fludrocortisone acetate. Its nystatin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of nystatin that it is represented to contain. Its neomycin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of neomycin that it is represented to contain. Its polymyxin B content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of polymyxin B that it is represented to contain. Its pH is not less than 4.0 and not more than 6.0. The nystatin used conforms to the standards prescribed by § 449.50(a)(1), except safety. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1), except safety, of this chapter. The polymyxin B sulfate used conforms to the standards prescribed by § 448.30a(a)(1), except safety, of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(b) The neomycin sulfate used in making the batch for potency, loss on drying, pH, and identity.

(c) The polymyxin B sulfate used in making the batch for potency, loss on drying, residue on ignition, pH, and identity.

(d) The batch for potency, loss on drying, and pH.

(ii) Samples required:

(a) The nystatin used in making the batch: 10 packages, each consisting of not less than 300 milligrams.

(b) The neomycin sulfate used in making the batch: 10 packages, each consisting of not less than 300 milligrams.

(c) The polymyxin B sulfate used in making the batch: 10 packages, each consisting of not less than 300 milligrams.

(d) The batch: A minimum of seven immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Nystatin.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Remove an accurately measured representative portion and dilute with sufficient dimethylformamide to give a stock solution containing 400 units of nystatin per milliliter (estimated). Further dilute with 10 percent potassium phosphate buffer, pH 6 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(ii) *Neomycin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling, remove an accurately measured representative portion, and dilute with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(iii) *Polymyxin B content.* Proceed as directed in § 436.105 of this chapter, except add to each concentration of the polymyxin standard curve a quantity of neomycin to yield the same concentration of neomycin as that present when the sample is diluted to contain 10 units of polmyxin B per milliliter. Prepare the sample for assay as follows: Reconstitute as directed in the labeling, remove an accurately measured representative portion, and dilute with sufficient 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to give a stock solution of convenient concentration. Further dilute with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter, using the dry powder.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in its labeling.

Subpart F—Dermatologic Dosage Forms

§ 449.504 Amphotericin B dermatologic dosage forms.

§ 449.504a Amphotericin B ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Amphotericin B ointment is composed of amphotericin B in a suitable and harmless ointment base. It may contain suitable and harmless coloring agents and protectants. It contains 30 milligrams of amphotericin B in each gram. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of amphotericin B that it is represented to contain. Its moisture content is not more than 1.0 percent. The amphotericin B used conforms to the standards prescribed by § 449.4(a)(1)(i), (ii), (v), (vi), and (vii).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of

§ 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The amphotericin B used in making the batch for potency, amphotericin A content, pH, residue on ignition, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) Amphotericin B used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample (usually 1 gram) into an appropriate-sized Erlenmeyer flask with 10 milliliters of ethyl ether. Allow to dissolve for 1 hour with intermittent manual shaking. Add a measured amount of dimethylsulfoxide to the flask and place on a shaker for 10 minutes. Further dilute with dimethylsulfoxide to a concentration of 20 micrograms of amphotericin B per milliliter (estimated). Remove an aliquot and dilute with 0.2*M* potassium phosphate buffer, pH 10.5 (solution 10), to the reference concentration of 1.0 microgram of amphotericin B per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 449.504b Amphotericin B cream.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Amphotericin B cream is composed of amphotericin B, with or without one or more suitable and harmless emollients, perfumes, dispersants, and preservatives, in a suitable and harmless cream base. It contains 30 milligrams of amphotericin B in each gram. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of amphotericin B per gram that it is represented to contain. The amphotericin B used conforms to the standards prescribed by § 449.4 (a) (1) (i), (ii), (v), (vi), and (vii).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The amphotericin B used in making the batch for potency, amphotericin A content, pH, residue on ignition, and identity.

(*b*) The batch for potency.

(ii) Samples required:

(*a*) Amphotericin B used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay; potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: With the aid of a high-speed glass blender, dissolve an accurately weighed sample in sufficient dimethylsulfoxide to give a stock solution of convenient concentration. Further dilute with dimethylsulfoxide to a concentration of 20 micrograms of amphotericin B per milliliter (estimated). Remove an aliquot and dilute with 0.2*M* potassium phosphate buffer, pH 10.5 (solution 10), to the reference concentration of 1.0 microgram of amphotericin B per milliliter (estimated).

§ 449.504c Amphotericin B lotion.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Amphotericin B lotion is composed of amphotericin B in a suitable and harmless lotion vehicle. It contains suitable and harmless emollients, emulsifiers, coloring agents, diluents, preservatives, and perfumes. It contains 30 milligrams of amphotericin B per milliliter. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of amphotericin B per milliliter that it is represented to contain. Its pH is not less than 5.0 and not more than 7.0. The amphotericin B used conforms to the standards prescribed by § 449.4 (a) (1) (i), (ii), (v), (vi), and (vii).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The amphotericin B used in making the batch for potency, amphotericin A content, pH, residue on ignition, and identity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The amphotericin B used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an aliquot in sufficient dimethylsulfoxide to give a stock solution of convenient concentration. Further dilute the stock solution with dimethylsulfoxide to a concentration of 20 micrograms of amphotericin B per milliliter (estimated). Remove an aliquot and dilute with 0.2*M* potassium phosphate buffer, pH 10.5 (solution 10), to the reference concentration of 1.0 microgram of amphotericin B per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted lotion.

§ 449.550 Nystatin dermatologic dosage forms.

§ 449.550a Nystatin ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin ointment is composed of nystatin and a suitable and harmless ointment base. Each gram contains 100,000 units of nystatin. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of nystatin that it is represented to contain. The moisture content is not more than 0.5 percent. The nystatin used conforms to the standards prescribed by § 449.50(a) (1) (i), (iii), (iv), and (v).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 containers, each consisting of 300 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Using sufficient dimethylformamide to give a concentration of 400 units of nystatin (estimated) per milliliter, blend an accurately weighed representative portion in a high-speed glass blender for 3 to 5 minutes. Further dilute with 10 percent potassium phosphate buffer, pH 6 (solution 6), to the referenee concentration of 20 units of nystatin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 449.550b Nystatin-iodochlorhydroxyquin ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin-iodochlorhydroxyquin ointment is composed of nystatin and iodochlorhydroxyquin in a suitable and harmless ointment base. Each gram contains 100,000 units of nystatin and 10 milligrams of iodochlorhydroxyquin. Its nystatin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of nystatin that it is represented to contain. Its iodochlorhydroxyquin content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of iodochlorhydroxyquin that it is represented to contain. It passes the identity test for iodochlorhydroxyquin. Its moisture content is not more than 0.5 percent. The nystatin used conforms to the standards prescribed by § 449.50 (a) (1). The iodochlorhydroxyquin used conforms to the standards prescribed by U.S.P. XVIII.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, safety, loss on drying, pH, and identity.

(*b*) The iodochlorhydroxyquin used in making the batch for all U.S.P. XVIII specifications.

(*c*) The batch for nystatin content, iodochlorhydroxyquin content, iodochlorhydroxyquin identity, and moisture.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of seven immediate containers.

(b) *Tests and methods of assay*—(1) *Nystatin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a high-speed glass blender jar containing sufficient dimethylformamide to give a convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and dilute with sufficient dimethylformamide to yield a stock solution containing 400 units of nystatin per milliliter (estimated). Further dilute an aliquot of the stock solution with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(2) *Iodochlorhydroxyquin content*—(i) *Reagents.* (*a*) Ferric chloride reagent. Dissolve 1.0 gram of ferric chloride ($FeCl_3 \cdot 6H_2O$) in a mixture of 1.0 milliliter of concentrated hydrochloric acid and sufficient distilled water to make 1 liter.

(*b*) Acetone, reagent grade.

(*c*) 2-Methoxyethanol, reagent grade.

(ii) *Preparation of standard solution.* Dissolve an accurately weighed portion of iodochlorhydroxyquin U.S.P. reference standard in sufficient 2-methoxyethanol to make a solution containing 1.0 milligram of iodochlorhydroxyquin per milliliter. Transfer 5.0 milliliters of this standard solution to a 50-milliliter volumetric flask.

(iii) *Preparation of sample solution.* Accurately weigh a portion of the sample equivalent to 50 milligrams of iodochlorhydroxyquin into a 125-milliliter Erlenmeyer flask. Add 50 milliliters of acetone, warm on a steam bath, and shake gently. Cool to room temperature and filter contents through a pledget of glass wool into a 100-milliliter volumetric flask. Wash the Erlenmeyer flask with two 20-milliliter portions of acetone and filter the washings into the volumetric flask. Dilute to volume with acetone and mix thoroughly. Transfer a 10-milliliter aliquot of the acetone solution to a 50-milliliter volumetric flask and evaporate on a steam bath. To the residue, add 20 milliliters of 2-methoxyethanol and swirl to dissolve the iodochlorhydroxyquin.

(iv) *Procedure.* To each flask containing standard solution and sample solution, respectively, add 2.0 milliliters of ferric chloride reagent and dilute to volume with 2-methoxyethanol. Mix thoroughly. Using a suitable spectrophotometer equipped with 1.0-centimeter cells and a blank prepared by diluting 2.0 milliliters of ferric chloride reagent to 50 milliliters with 2-methoxyethanol, determine the absorbance of the sample and standard solutions at 650 nanometers. Set the instrument to 100-percent transmission with the blank.

(v) *Calculation.*

$$\text{Milligrams of iodochlorhydroxyquin per gram of sample} = \frac{\text{Absorbance of sample} \times 50}{\text{Absorbance of standard} \times \text{Weight of sample in grams}}$$

(3) *Iodochlorhydroxyquin identity.* Proceed as directed in § 436.400 of this chapter, preparing the sample solution as follows: Accurately weigh a portion of the sample equivalent to 50 milligrams of iodochlorhydroxyquin into a 125-milliliter Erlenmeyer flask. Add 50 milliliters of acetone, warm on a steam bath, and shake gently. Cool to room temperature and filter contents through a pledget of glass wool into a 100-milliliter volumetric flask. Wash the Erlenmeyer flask with two 20-milliliter portions of acetone and filter the washings into the volumetric flask. Dilute to volume with acetone and mix thoroughly.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 449.550c Nystatin-neomycin sulfate-gramicidin-triamcinolone acetonide ointment; nystatin-neomycin sulfate-gramicidin-fludrocortisone acetate ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* The drug is nystatin, neomycin sulfate, gramicidin, and either triamcinolone acetonide or fludrocortisone acetate in a suitable ointment base. Each gram contains 100,000 units of nystatin, 2.5 milligrams of neomycin, 0.25 milligram of gramicidin, and either 1.0 milligram of triamcinolone acetonide or 1.0 milligram of fludrocortisone acetate. Its nystatin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of nystatin that it is represented to contain. Its neomycin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of neomycin that it is represented to contain. Its gramicidin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of gramicidin that it is represented to contain. Its moisture content is not more than 0.5 percent. The nystatin used conforms to the standards prescribed by § 449.50(a)(1) (i), (iii), (iv), and (v). The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1) except safety, of this chapter. The gramicidin used conforms to the standards prescribed by § 448.25(a)(1) (i), (iii), (iv), (v), and (vi) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The neomycin sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*c*) The gramicidin used in making the batch for potency, loss on drying, residue on ignition, melting point, crystallinity, and identity.

(*d*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each consisting of 300 milligrams.

(*b*) The neomycin sulfate used in making the batch: 10 packages, each consisting of 300 milligrams.

(*c*) The gramicidin used in making the batch: 10 packages, each consisting of 500 milligrams.

(*d*) The batch: A minimum of seven immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Nystatin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Blend an accurately weighed representative portion in a high-speed glass blender for 3 to 5 minutes with sufficient dimethylformamide to give a concentration of 400 units of nystatin per milliliter (estimated). Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(ii) *Neomycin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the ointment into a separatory funnel containing 50 milliliters of peroxide-free ether. Shake the sample and ether until homogenous. Add 20 to 25 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction with new portions of the buffer at least three times and any additional times necessary to insure complete extraction of the antibiotic. Combine the extractives and adjust to an appropriate volume to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(iii) *Gramicidin content.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Accurately weigh and dissolve a representative portion of the sample in 50 milliliters of petroleum ether in a separatory funnel. Extract with 20 milliliters of 80 percent ethyl alcohol. Repeat the extraction three times. Pool the extractives in a suitable volumetric flask and bring to volume with 80 percent ethyl alcohol. Further dilute with 95 percent ethyl alcohol to the reference concentration of 0.04 microgram of gramicidin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 449.550d Nystatin cream.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin cream is composed of nystatin and suitable and harmless emulsifiers, perfumes, buffers, preservatives, and a protectant in a suitable and harmless cream base. Each gram contains 100,000 units of nystatin. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of nystatin that it is represented to contain. The nystatin used conforms to the standards prescribed by § 449.50(a)(1)(i), (iii), (iv), and (v).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(b) The batch for potency.

(ii) Samples required:

(a) The nystatin used in making the batch: 10 containers, each consisting of 300 milligrams.

(b) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay; potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Using sufficient dimethylformamide to give an estimated concentration of 400 units of nystatin per milliliter, blend an accurately weighed representative portion in a high-speed blender for 3 to 5 minutes. Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

§ 449.550e Nystatin-neomycin sulfate-gramicidin-triamcinolone acetonide cream.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin-neomycin sulfate-gramicidin-triamcinolone acetonide cream is composed of nystatin, neomycin sulfate, gramicidin, triamcinoline acetonide, and suitable and harmless emulsifiers, solvents, perfumes, buffers, preservatives, and a protectant in a suitable cream base. Each gram contains 100,000 units of nystatin, 2.5 milligrams of neomycin, 0.25 milligram of gramicidin, and 1 milligram of triamcinolone acetonide. Its nystatin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of nystatin that it is represented to contain. Its neomycin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of neomycin that it is represented to contain. Its gramicidin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of gramicidin that it is represented to contain. The nystatin used conforms to the standards prescribed by § 449.50(a)(1) (i), (iii), (iv), and (v). The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1), except safety, of this chapter. The gramicidin used conforms to the standards prescribed by § 448.25 (a)(1) (i), (iii), (iv), (v), and (vi) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(b) The neomycin sulfate used in making the batch for potency, loss on drying, pH, and identity.

(c) The gramicidin used in making the batch for potency, loss on drying, residue on ignition, melting point, crystallinity, and identity.

(d) The batch for nystatin content, neomycin content, and gramicidin content.

(ii) Samples required:

(a) The nystatin used in making the batch: 10 packages, each consisting of 300 milligrams.

(b) The neomycin sulfate used in making the batch: 10 packages, each consisting of 300 milligrams.

(c) The gramicidin used in making the batch: 10 packages, each consisting of 500 milligrams.

(d) The batch: A minimum of seven immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Nystatin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Using sufficient dimethylformamide to give a concentration of 400 units of nystatin (estimated) per milliliter, blend an accurately weighed representative portion in a high-speed glass blender for 3 to 5 minutes. Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(ii) *Neomycin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the cream in a separatory funnel containing 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction with new portions of the buffer at least three times and any additional times necessary to ensure complete extraction of the antibiotic. Combine the extractives and adjust to an appropriate volume to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(iii) *Gramicidin content.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Accurately weigh a representative portion of the sample and dissolve in 50 milliliters of petroleum ether in a separatory funnel. Extract with 20 milliliters of 80 percent ethyl alcohol. Repeat the extraction three times. Pool the extracts in a suitable volumetric flask and bring to mark with 80 percent ethyl alcohol. Further dilute with 95 percent ethyl alcohol to the reference concentration of 0.04 microgram of gramicidin per milliliter (estimated).

§ 449.550f Nystatin topical powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin topical powder is a dry powder composed of nystatin and talc. Each gram contains 100,000 units of nystatin. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of nystatin that it is represented to contain. Its loss on drying is not more than 2.0 percent. The nystatin used conforms to the standards prescribed by § 449.50(a)(1) (i), (iii), (iv), and (v).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(b) The batch for potency and loss on drying.

(ii) Samples required:

(a) The nystatin used in making the batch: 10 packages, each containing 300 milligrams.

(b) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Blend an accurately weighed representative sample for 3 to 5 minutes in a high-speed glass blender with sufficient dimethylformamide to give a convenient concentration. Dilute with sufficient dimethylformamide to yield a stock solution containing 400 units of nystatin per milliliter (estimated). Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

§ 449.550g Nystatin-neomycin sulfate-gramicidin topical powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin-neomycin sulfate-gramicidin topical powder is a dry powder composed of nystatin, neomycin sulfate, gramicidin, and talc. Each gram contains 100,000 units of nystatin,

2.5 milligrams of neomycin and 0.25 milligram of gramicidin. Its nystatin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of nystatin that it is represented to contain. Its neomycin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of neomycin that it is represented to contain. Its gramicidin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of gramicidin that it is represented to contain. Its loss on drying is not more than 2.0 percent. The nystatin used conforms to the standards prescribed by § 449.50(a)(1) (i), (iii), (iv), and (v). The neomycin sulfate used conforms to the standards prescribed by § 442.42a(a)(1), except safety, of this chapter. The gramicidin used conforms to the standards prescribed by § 448.25(a)(1) (i), (iii), (iv), (v) and (vi) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The neomycin sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*c*) The gramicidin used in making the batch for potency, loss on drying, residue on ignition, melting point, crystallinity, and identity.

(*d*) The batch for nystatin content, neomycin content, gramicidin content, and loss on drying.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages each consisting of 300 milligrams.

(*b*) The neomycin sulfate used in making the batch: 10 packages, each consisting of 300 milligrams.

(*c*) The gramicidin used in making the batch: 10 packages, each consisting of 500 milligrams.

(*d*) The batch: A minimum of seven immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Nystatin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Blend the entire contents of an accurately weighed representative portion of the sample for 3 to 5 minutes in a high-speed glass blender with sufficient dimethylformamide to give a convenient concentration. Dilute with sufficient dimethylformamide to yield a stock solution containing 400 units of nystatin per milliliter (estimated). Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(ii) *Neomycin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Blend an accurately weighed representative sample for 3 to 5 minutes in sufficient 0.1*M* potassium phosphate buffer, pH 8 (solution 3), to give a convenient concentration. Further dilute an aliquot with solution 3 to the reference concentration of 1 microgram of neomycin per milliliter (estimated).

(iii) *Gramicidin content.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed representative sample in 95 percent ethyl alcohol and filter. Collect the filtrate and dilute a portion with 95 percent ethyl alcohol to the reference concentration of 0.04 microgram of gramicidin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

Subpart G—Vaginal Dosage Forms

§ 449.610 Candicidin vaginal dosage forms.

§ 449.610a Candicidin vaginal ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Candicidin vaginal ointment is composed of candicidin and a suitable ointment base. It contains 0.6 milligram of candicidin per gram. Its potency is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of candicidin that it is represented to contain. Its moisture content is not more than 0.1 percent. The candicidin used conforms to the requirements of § 449.10(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The candicidin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The candicidin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Accurately weigh approximately 3 grams of the ointment into a 50-milliliter centrifuge tube. Dissolve the ointment in 30 milliliters of *n*-hexane by warming the centrifuge tube with hot water. Centrifuge for 15 minutes at 2,000 revolutions per minute. Carefully decant the supernatant, leaving all sediment in the tube. Repeat the suspending and centrifuging operation three times or until all the ointment base is washed out. After the final wash, evaporate the *n*-hexane by air evaporation. Dissolve the sediment in 10 milliliters of dimethylsulfoxide, quantitatively transfer to a sterile 200-milliliter volumetric flask, and fill to volume with sterile distilled water. Further dilute with sterile distilled water to the reference concentration of 0.06 microgram of candicidin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 449.610b Candicidin vaginal tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Candicidin vaginal tablets are tablets composed of candicidin with suitable binders, diluents, and lubricants. Each tablet contains 3 milligrams of candicidin. Its potency is satisfactory if it is not less than 90 percent and not more than 160 percent of the number of milligrams of candicidin that it is represented to contain, except that for the issuance of a certificate for each batch, the candicidin content must be not less than 115 percent and not more than 150 percent of the number of milligrams of candicidin that it is represented to contain. The tablets shall disintegrate within 30 minutes. The loss on drying is not more than 1 percent. The candicidin used in making the batch conforms to the standards of § 449.10(a)(1).

(2) *Labeling.* The drug shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The candicidin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency, loss on drying, and disintegration time.

(ii) *Samples required.* (*a*) The candicidin used in making the batch: 10 packages, each consisting of approximately 300 milligrams.

(*b*) The batch: A minimum of 56 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Weigh a pool of five tablets and grind in a mortar to a very fine powder. Suspend an accurately weighed aliquot (of approximately 2 grams) in 10 milliliters of dimethylsulfoxide. Centrifuge for 5 minutes at 2,000 revolutions per minute. Carefully decant the supernatant solution into a sterile 250-milliliter volumetric flask. Wash the residue three times with 5-milliliter portions of dimethylsulfoxide, centrifuging each time. Add the washes to the 250-milliliter volumetric flask and fill to volume with sterile distilled water. Using sterile distilled water, further dilute to the reference concentration of 0.06 microgram of candicidin per milliliter (estimated).

(2) *Disintegration time.* Proceed as directed in § 436.212 of this chapter, using the method described in paragraph (e)(1) of that section, except use distilled water as the immersion fluid.

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

§ 449.610c Candicidin vaginal capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength,*

quality, and purity. Candicidin vaginal capsules are gelatin capsules containing 3 milligrams of candicidin in a suitable and harmless ointment. The candicidin content is satisfactory if it is not less than 90 percent and not more than 150 percent of the number of milligrams of candicidin that it is represented to contain. The moisture content is not more than 0.1 percent. The candicidin used conforms to the requirements of § 449.10(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The candicidin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The candicidin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 20 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, except prepare the sample as follows: Remove tips from two capsules and express the ointment from each capsule into one 50-milliliter glass-stoppered centrifuge tube. Wash out the capsules with warm *n*-hexane adding the washes to the 50-milliliter centrifuge tube. Completely dissolve the ointment base in the *n*-hexane by warming the centrifuge tube with hot water, if necessary. Centrifuge for 15 minutes at 2,000 revolutions per minute. Carefully decant the supernatant leaving all sediment in the tube. Repeat the suspending and centrifuging operations three times or until all ointment base is washed out. After the final wash, evaporate the *n*-hexane by air evaporation. Dissolve the sediment in 20 milliliters of dimethylsulfoxide, quantitatively transfer to a sterile 250-milliliter volumetric flask, and fill to volume with sterile distilled water. Further dilute with sterile distilled water to the reference concentration of 0.06 microgram of candicidin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 449.650 Nystatin vaginal tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin vaginal tablets are tablets composed of nystatin and suitable and harmless diluents, binders, and lubricants. Each tablet contains 100,000 units of nystatin. Its potency is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of nystatin that it is represented to contain. The loss on drying is not more than 5 percent. The disintegration time is not more than 1 hour. The nystatin used conforms to the standards prescribed therefor by § 449.50(a)(1) (i), (iii), (iv), and (v).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for nystatin content, loss on drying, and disintegration time.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 immediate containers of approximately 300 milligrams each.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Blend a representative number of tablets for 3 to 5 minutes in a high-speed glass blender with sufficient dimethylformamide to give a convenient concentration. Dilute an aliquot with sufficient dimethylformamide to give a stock solution containing 400 units of nystatin per milliliter (estimated). Further dilute the stock solution with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter, using the procedure described in paragraph (e)(1) of that section, except use distilled water in lieu of gastric fluid.

PART 450—ANTITUMOR ANTIBIOTIC DRUGS

Subpart A—Bulk Drugs

450.20a Sterile dactinomycin.
450.40 Mithramycin.
450.45 Mitomycin.

Subpart B—[Reserved]

Subpart C—Injectable Dosage Forms

450.220 Dactinomycin for injection.
450.240 Mithramycin for injection.
450.245 Mitomycin for injection.

AUTHORITY: Sec. 507, 59 Stat. 463, as amended (21 U.S.C. 357).

Subpart A—Bulk Drugs

§ 450.20a Sterile dactinomycin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Dactinomycin is a bright-red compound that is so purified and dried that:

(i) Its potency is not less than 900 micrograms of dactinomycin per milligram, calculated on an anhydrous basis.

(ii) It is sterile.

(iii) It is pyrogen-free.

(iv) Its LD_{50}[*] in mice is not less than 0.65 and not more than 1.23 milligrams of dactinomycin per kilogram of body weight.

(v) Its loss on drying is not more than 15 percent.

(vi) Its absorptivity at 445 nanometers is not less than 0.95 and not more than 1.03 times that of the dactinomycin working standard at the same wavelength. Its absorbance at 240 nanometers is not less than 1.3 and not more than 1.5 times its absorbance at 445 nanometers.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter, and in addition each package shall bear on its label the statement "Protect from light and excessive heat".

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, LD_{50}, loss on drying, absorptivity, and crystallinity.

(ii) Samples required:

(*a*) For all tests except sterility: 16 packages, each containing approximately 40 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 40 milligrams.

(b) *Tests and methods of assay.* Dactinomycin is toxic and corrosive. It must be handled with care in the laboratory. Transfer all dry powders in a suitable hood, while wearing rubber gloves. Avoid inhaling fine particles of the powder. Do not pipette by mouth. If any of the substance contacts the skin, wash copiously with soap and water. Dispose of all waste material by dilution with large volumes of trisodium phosphate solution.

(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient methyl alcohol to give a stock solution of 10 milligrams of dactinomycin per milliliter (estimated). Further dilute the stock solution with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of dactinomycin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use 40 milligrams in lieu of 300 milligrams.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 0.2 milligram of dactinomycin per milliliter.

(4) LD_{50}—(i) *Sample solution.* Prepare a solution containing 50 micrograms of dactinomycin per milliliter by dissolving an appropriate quantity of the sample in sufficient sterile distilled water. If the sample does not dissolve immediately, place it in an ice water bath or a refrigerator for 1 to 2 hours, and then allow to reach room temperature before use.

(ii) *Procedure.* Select 70 female mice weighing between 18 and 20 grams. Individually identify and weigh them to the nearest 0.1 gram. Use 10 mice at each of the following dose levels: 9.6,

[*] The term "LD_{50}" refers to the dosage of the drug that should be expected to kill 50 percent of the animals that receive the drug.

11.52, 13.9, 16.7, 20.0, 24.0, and 28.8 milliliters of sample solution per kilogram of body weight. Calculate the volume of solution to be given to each mouse and administer the appropriate volume intravenously at the rate of 0.5 milliliter per minute. Observe the mice daily for 14 days and record any signs of drug toxicity and times of death. Estimate the LD_{50} and its 95 percent confidence limits by the method of Carrol S. Weil, published in *Biometrics*, volume 8, pages 249–263 (1952).[1]

(5) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(6) *Absorptivity*—(i) *Procedure.* Accurately weigh approximately 15 milligrams of the sample "as is" and 15 milligrams of the working standard dried as directed in § 436.200(a) of this chapter. Transfer each weighing to separate 100-milliliter volumetric flasks. Dissolve the material and bring to volume with spectrophotometric-grade methyl alcohol. Mix well. Pipette 5.0 milliliters of each solution into separate 25-milliliter volumetric flasks, dilute to volume with spectrophotometric-grade methyl alcohol. Mix well. Using a suitable spectrophotometer and 1-centimeter absorption cells, determine the absorbance of the sample solution at the 240-nanometer and at the 445-nanometer absorption peaks (the exact position of the peaks should be determined for the particular instrument used). Determine the absorbance of the standard at the 445-nanometer absorption peak.

(ii) *Calculations.* Calculate the relative absorptivity and the ratio for the absorbances of the sample as follows:

$$\text{Relative absorptivity at 445 nanometers} = \frac{A_3 \times \text{milligrams of standard} \times \text{potency of the standard in micrograms per milligram}}{A_2 \times \text{milligrams of sample} \times (100 - M) \times 10}$$

$$\text{Ratio for the absorbances of the sample at 240 and 445 nanometers} = \frac{A_1}{A_2}$$

where:

A_1 = Absorbance at 240 nanometers for the sample.
A_2 = Absorbance at 445 nanometers for the sample.
A_3 = Absorbance at 445 nanometers for the standard.
M = Percent moisture in the sample.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

§ 450.40 Mithramycin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Mithramycin is a yellow compound and is so purified and dried that:

(i) Its potency is not less than 900 micrograms of mithramycin per milligram calculated on an anhydrous basis.

(ii) Its loss on drying is not more than 8 percent.

(iii) Its pH in an aqueous solution containing 0.5 milligram per milliliter is not less than 4.5 nor more than 5.5.

(iv) Its absorptivity on the anhydrous basis at the absorption maximum of 278 millimicrons is 100±5 percent of that of the mithramycin standard similarly treated.

(v) It gives a positive result to the identity tests for mithramycin.

(vi) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. In addition, each package shall bear on its label the statement "Store below 10° C. (50° F.)".

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, absorptivity, identity, and crystallinity.

(ii) Samples required on the batch: 2 packages, each containing not less than 100 milligrams; and 2 packages, each containing not less than 50 milligrams.

(b) *Tests and methods of assay.* Mithramycin is more toxic than the average drug and must be handled with care in the laboratory. Avoid inhaling fine particles of powder. If the substance contacts the skin, wash with soap and water. Solutions should not be pipetted by mouth. Mithramycin is hygroscopic and care should be exercised during storage and weighing samples. Samples should be stored at 10° C. or less in a sealed, light-resistant container with a desiccant. Dispose of all waste material by dilution with larger volumes of trisodium phosphate solution.

(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to give a stock solution of convenient concentration. Further dilute the stock solution with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 1.0 microgram of mithramycin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(g) of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 0.5 milligram of mithramycin per milliliter. Allow the solution to remain in contact with the electrodes until a steady reading is obtained or for 5 minutes.

(4) *Absorptivity.* Determine the absorbance of the sample and standard solutions in the following manner: Dissolve approximately 10 milligrams each of the sample and standard (dried as described in § 436.200(g) of this chapter), accurately weighed, in 50 milliliters of absolute methanol. Transfer 5-milliliter portions into 100-milliliter volumetric flasks and dilute to volume with 0.01*N* hydrochloric acid in methanol prepared by diluting 20 milliliters of 0.5*N* aqueous hydrochloric acid to 1 liter with absolute methanol. Using a suitable spectrophotometer and 0.01*N* hydrochloric acid in methanol as the blank, scan the absorption spectrum between the wavelengths of 220 millimicrons and 400 millimicrons. Determine the absorbance of each solution at the absorption maximum near 278 millimicrons. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculation:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample solution} \times \text{milligrams of standard} \times \text{potency of standard in micrograms per milligram}}{\text{Absorbance of standard solution} \times \text{milligrams of sample} \times 10}$$

(5) *Identity.* (i) The absorption spectrum between the wavelengths of 220 and 400 millimicrons determined as directed in paragraph (b)(4) of this section compares qualitatively with that of the mithramycin standard, and the ratio of the absorbance at 278 millimicrons to the absorbance at 228 millimicrons is 100±5 percent of that of the mithramycin standard similarly treated.

(ii) Proceed as directed in § 436.211 of this chapter, using a 0.25 percent mixture of the sample in a potassium bromide disc prepared as directed in paragraph (b)(1) of that section.

(6) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

§ 450.45 Mitomycin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Mitomycin is 7-amino-9a-methoxymitosane. It is a blue-violet compound that is soluble in water, methanol, acetone, butyl acetate, and cyclohexanone. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram.

(ii) It passes the safety test.

(iii) Its moisture content is not more than 5 percent.

(iv) Its pH in a solution containing 5 milligrams per milliliter is not less than 6.0 and not more than 8.0.

(v) When calculated on the anhydrous basis, its absorptivity at 357 nanometers is not less than 95 percent and not more than 105 percent of that of the mitomycin working standard similarly treated.

(vi) It gives a positive identity test for mitomycin.

(vii) It is crystalline.

[1] Copies may be obtained from: Managing Editor, "Biometrics", P.O. Box 5457, Raleigh, NC 27607.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, moisture, pH, absorptivity, identity, and crystallinity.

(ii) Samples required: Five packages, each containing approxmiately 100 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 microgram of mitomycin per milliliter (estimated).

(2) *Safety.* Proceed as directed in § 436.33 of this chapter, except observe the mice for 7 days in lieu of 48 hours, noting mortality every 24 hours. Repeat the test as described in § 436.33(d) of this chapter if one or more animals die within 7 days.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution containing 5 milligrams per milliliter.

(5) *Absorptivity.* Determine the absorbance of the sample and standard solution in the following manner: Place an accurately weighed portion of approximately 25 milligrams of mitomycin into a 50-milliliter volumetric flask. Dissolve and dilute to volume with absolute methanol. Further dilute an aliquot with absolute methanol to 0.005 milligram of mitomycin per milliliter. Using a suitable spectrophotometer equipped with a 1-centimeter quartz cell and absolute methanol as the blank, determine the absorbance of the sample and standard solutions at 357 nanometers. Calculate the percent relative absorptivity as follows:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{weight of standard in milligrams} \times \text{percent mitomycin content of standard} \times 100}{\text{Absorbance of standard} \times \text{weight of sample in milligrams} \times (100-m)}$$

where:
m = percent moisture in the sample.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b)(2) of that section.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

Subpart B—[Reserved]

Subpart C—Injectable Dosage Forms

§ 450.220 Dactinomycin for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Dactinomycin for injection is a dry mixture of dactinomycin and mannitol. Each container contains 0.5 milligram of dactinomycin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of dactinomycin that it is represented to contain. It is sterile. It is nonpyrogenic. The LD_{50}[a] in mice is between 0.65 and 1.23 milligrams of dactinomycin per kilogram. Its loss on drying is not more than 4.0 percent. Its pH is not less than 5.5 and not more than 7.0. The crystalline dactinomycin used conforms to the standards prescribed by § 450.20a (a)(1)(i), (v), and (vi).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter, and in addition each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the outside wrapper or container the statement "Protect from light and excessive heat".

(ii) On the outside wrapper or container and the immediate container the statement "For hospitalized patients only".

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The dactinomycin used in making the batch: For potency, loss on drying, absorptivity, and crystallinity.

(*b*) The batch: For microbiological potency, spectrophotometric potency, sterility, pyrogens, LD_{50}, loss on drying, and pH.

(ii) Samples required:

(*a*) The dactinomycin used in making the batch: 10 containers each containing not less than 40 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers.

(b) *Tests and methods of assay.* Dactinomycin is toxic and corrosive. It must be handled with care in the laboratory. Transfer all dry powders in a suitable hood, while wearing rubber gloves. Avoid inhaling fine particles of the powder. Do not pipette by mouth. If any of the substance contacts the skin, wash copiously with soap and water. Dispose of all waste material by dilution with large volumes of trisodium phosphate solution.

(1) *Potency*—(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute the product as directed in the labeling by the addition of 1.1 milliliters of sterile water for injection. Withdraw a 1.0-milliliter aliquot, using a suitable hypodermic needle and syringe, and dilute with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of dactinomycin per milliliter (estimated).

(ii) *Spectrophotometric assay*—(*a*) *Preparation of standard solution.* Accurately weigh approximately 15 milligrams of the working standard dried as directed in § 436.200(a) of this chapter and transfer to a 100-milliliter volumetric flask. Dissolve the standard and bring to volume with spectrophotometric-grade methyl alcohol. Mix well. Pipette 5.0 milliliters into a 25-milliliter volumetric flask and dilute to volume with spectrophotometric-grade methyl alcohol.

(*b*) *Preparation of sample solution.* Reconstitute the product as directed in the labeling by the addition of 1.1 milliliters of sterile water for injection. Withdraw a 1.0-milliliter aliquot using a suitable hypodermic needle and syringe and dilute the sample with sufficient spectrophotometric-grade methyl alcohol to obtain a concentration of 20 micrograms of dactinomycin per milliliter (estimated). Obtain a clear solution by decanting or filtering out any undissolved mannitol.

(*c*) *Procedure.* Determine the absorbance of the sample and standard solution at the 445-nanometer absorption peak as directed in § 450.20a(b)(6)(i).

(*d*) *Calculation:*

$$\text{Milligrams of dactinomycin per milliliter} = \frac{A_1 \times \text{milligrams of standard weighed} \times \text{potency of standard in micrograms per milligram} \times \text{milliliters of methyl alcohol sample solution}}{A_2 \times 500 \times 1{,}000}$$

where:
A_1 = Absorbance of the sample solution at 445 nanometers.
A_2 = Absorbance of the standard solution at 445 nanometers.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use the entire contents of each of the immediate containers tested.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, preparing the sample for test as follows: Use a sufficient number of containers to yield 3 milligrams of dactinomycin. Reconstitute by adding 1.1 milliliters of sterile water for injection to each container. Aseptically pool the resultant solutions from each container. Dilute an accurately measured portion with sufficient diluent 1 to give a concentration of 0.2 milligram of dactinomycin per milliliter.

[a] The term "LD_{50}" refers to the dosage of the drug that should be expected to kill 50 percent of the animals that receive the drug.

(4) *LD_m.* Proceed as directed in § 450.20a(b)(4), except prepare the sample for test as follows: Reconstitute a sufficient number of containers to yield 2,000 micrograms of dactinomycin. Reconstitute by adding 1.1 milliliters of sterile water for injection to each container. Aseptically pool the resultant solutions from each container. Dilute an accurately measured portion with sufficient sterile distilled water to give a concentration of 50 micrograms of dactinomycin per milliliter (estimated). Use the potency value obtained in § 450.220(b)(1) in calculating the LD_m value.

(5) *Loss on drying.* Proceed as directed in § 436.200 of this chapter.

(6) *PH.* Reconstitute as directed in the labeling and proceed as directed in § 436.202 of this chapter.

§ 450.240 Mithramycin for injection.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Mithramycin for injection is a dry mixture of mithramycin and mannitol with or without a suitable buffer substance. Each immediate container contains 2.5 milligrams of mithramycin. Its potency is satisfactory if it contains not less than 90 percent and not more than 110 percent of the number of milligrams of mithramycin that it is represented to contain. It is sterile. It is nonpyrogenic. Its LD_m* in mice is not less than 1.2 and not more than 4.0 milligrams of mithramycin per kilogram of body weight. Its moisture content is not more than 2.0 percent. It contains no histamine nor histamine-like substances. Its pH when reconstituted as directed in the labeling is not less than 5.0 and not more than 7.5. It passes the identity test for mithramycin. The mithramycin used conforms to the standards prescribed by § 450.40(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. In addition, each package shall bear on its label or labeling the following as indicated:

(i) On the outside wrapper or container the statement "Store below 10° C. (50° F.)".

(ii) On the outside wrapper or container and on the immediate container the statement "Mandatory: Before using read enclosed professional information carefully for dosage instructions and warnings".

(iii) On the outside wrapper or container the statement "Warning: For hospital use only. To be used under direct supervision of a physician".

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The mithramycin used in making the batch for potency, loss on drying, absorptivity, pH, identity, and crystallinity.

(b) The batch for microbiological potency, spectrophotometric potency, sterility, pyrogens, LD_m, moisture, pH, histamine, and identity.

(ii) Samples required:

(a) The mithramycin used in making the batch: 3 packages, each containing not less than 50 milligrams; and 2 packages, each containing not less than 100 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 21 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay.* Mithramycin is more toxic than the average drug and must be handled with care in the laboratory. Avoid inhaling fine particles of powder. If the substance contacts the skin, wash with soap and water. Mithramycin is hygroscopic and care should be exercised during storage and weighing of samples. Dispose of all waste materials by dilution with larger volumes of trisodium phosphate solution. The samples should be stored at 10° C. or less in a sealed light-resistant container with a desiccant. Solutions should not be pipetted by mouth.

(1) *Potency—*(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Using a suitable syringe and hypodermic needle, withdraw the withdrawable contents from each container represented as a single-dose container or an accurately measured representative portion of the solution if represented in the labeling as containing a specified amount of antibiotic activity in a given volume. Dilute with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 1.0 microgram of mithramycin per milliliter (estimated).

(ii) *Spectrophotometric assay—*(a) *Preparation of standard solution.* Accurately weigh approximately 12 milligrams of the working standard (dried as described in § 436.200(g) of this chapter) and transfer to a 25-milliliter volumetric flask. Dissolve the standard and bring to volume with water. Mix well. Pipette 5.0 milliliters of this solution into a 250-milliliter volumetric flask, and dilute to volume with 0.01*N* hydrochloric acid in methanol prepared by diluting 20 milliliters of 0.5*N* aqueous hydrochloric acid to 1 liter with absolute methanol.

(b) *Preparation of sample solution.* Reconstitute the sample as directed in the labeling. Using a suitable syringe and hypodermic needle, withdraw the withdrawable contents from each container represented as a single-dose container or an accurately measured representative portion of the solution if represented in the labeling as containing a specified amount of antibiotic activity in a given volume. Dilute with sufficient 0.01*N* hydrochloric acid in methanol to obtain a concentration of approximately 10 micrograms of mithramycin per milliliter. Obtain a clear solution by decanting or filtering off any undissolved mannitol.

(c) *Procedure.* Determine the absorbance of the sample and standard solutions at the absorption peak at 278 millimicrons as directed in § 450.40(b)(4).

(d) *Calculations.*

$$\text{Milligrams of mithramycin per container} = \frac{A_1 \times \text{milligrams dry weight of standard} \times \text{potency of standard in micrograms per milligram}}{A_2 \times 5{,}000}.$$

where:

A_1 = Absorbance of the sample solution at 278 millimicrons;

A_2 = Absorbance of the standard solution at 278 millimicrons.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use the entire contents of each of the immediate containers tested.

(3) *Pyrogens.* Reconstitute the sample as directed in the labeling and proceed as directed in § 436.32 of this chapter, using a solution containing 50 micrograms of mithramycin per milliliter.

(4) *LD_m—*(i) *Sample solution.* Immediately prior to performing the LD_m test, dilute and pool a sufficient number of containers to yield 15.0 milligrams of mithramycin (estimated) with sufficient sterile distilled water to give a concentration of 0.8 milligram of mithramycin per milliliter (estimated). This solution is used for the maximum dose level (8.0 milligrams per kilogram of body weight) in this test. For lower dose levels, aliquots of this solution are diluted with sterile distilled water to concentrations of 0.4, 0.2, 0.1, and 0.05 milligram of mithramycin per milliliter, respectively. To determine the mithramycin content of the pooled solution used in the LD_m test, use the spectrophotometric assay described in paragraph (b)(1)(ii) of this section, except to prepare the sample solution, dilute an aliquot of the maximum dose level solution (0.8 milligram per milliliter) with sufficient 0.01*N* hydrochloric acid in methanol to obtain a concentration of approximately 10 micrograms of mithramycin per milliliter. Obtain a clear solution by decanting or filtering off any undissolved mannitol.

(ii) *Procedure.* Select 25 male and 25 female mice weighing between 18 and 22 grams each. These mice shall be conditioned for at least 48 hours prior to testing and shall be maintained on a diet of pellet food and water ad lib. Weigh them to the nearest gram. Use 5 males and 5 females at each of the following dose levels: 8.0, 4.0, 2.0, 1.0, and 0.5 milligrams mithramycin per kilogram of body weight. Inject each animal intravenously with a volume of 0.01 milliliter per gram of body weight and at a rate of 0.1 milliliter per second. Observe the mice daily for 10 days and record times of death. Estimate the combined sex LD_m of the pooled solution on the basis of its mithramycin content and its 95 percent confidence limits according to the method described in Cornfield and Mantel's modification of Karber's method

*The term "LD_m" refers to the dosage of the drug that should be expected to kill 50 percent of the animals that receive the drug.

published in the *Journal of the American Statistical Association*, vol. 45, pp. 193–210 (1950).[19] It passes the LD_m test if the combined sex LD_m in mice is between 1.2 and 3.0 milligrams of mithramycin per kilogram.

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter, using the total contents of three to five vials.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling. Allow the solution to remain in contact with the electrodes until a steady reading is obtained or for 5 minutes.

(7) *Histamine.* Proceed as directed in § 436.35 of this chapter.

(8) *Thin layer chromatography identity test for mithramycin*—(i) *Equipment*—(a) *Plates.* Use 20 by 20 centimeter or 15 by 20 centimeter thin layer chromatographic plates coated with Silica Gel Mixture, Chromatographic, U.S.P., to a thickness of 250 microns. Activate the plates by heating at 110° C. for 75 minutes. Place the plates in a desiccator until cooled to room temperature. Plates may be stored in a desiccator for 7 days.

(b) *Chamber (chromatographic).* A suitable chamber, equipped for thin layer chromatography.

(ii) *Preparations of solutions*—(a) *Solvent.* Mix reagent grade chloroform with reagent grade absolute methanol in volumetric proportions of 1:1.

(b) *Spray A.* Mix 50 milliliters of freshly prepared 1.0 percent ferric chloride in water (weight per volume), just before spraying, with 50 milliliters of freshly prepared 1.0 percent potassium ferricyanide in water (weight per volume).

(c) *Spray B.* Dissolve 2.28 grams of periodic acid in 100 milliliters of water. Dilute one volume of this periodic solution with 10 volumes of acetone.

(d) *Spray C.* Dissolve 184 milligrams of benzidine in a solution of 0.6 milliliter of acetic acid, 4.4 milliliters of water, and 95 milliliters of acetone.

(iii) *Preparation of spotting solutions*—(a) *Mithramycin standard solution.* Weigh 5 milligrams of mithramycin working standard and dissolve in 10 milliliters of methanol. Use the solution the same day it is prepared.

(b) *Mithramycin for injection sample solution.* Dilute with methanol to a concentration of 0.5 milligram of mithramycin per milliliter. Centrifuge and use the supernatant for spotting.

(c) *Mannitol reference solution.* Suspend 100 milligrams of mannitol in 5 milliliters of methanol. Centrifuge and use the supernatant for spotting.

(iv) *Procedure.* Fill the chamber to a depth of 0.6 centimeter with freshly prepared solvent. Spot duplicate plates as follows: On a line 2.5 centimeters from the base of the silica gel plate, and at intervals of 2.0 centimeters, spot 100 microliters (in four 25-microliter aliquots) of the standard solution, the sample solution, and the mannitol reference solution. Allow each aliquot to dry before applying subsequent volumes. After all spots are thoroughly dry, place the silica gel plates in the chromatographic chamber and develop by the ascending technique for approximately 60 minutes. Allow several minutes for the plates to air dry. On one plate, locate and record the position of fluorescent spots by examining under long wave ultraviolet light. Apply spray A and record the position of blue spots on the yellow-green background. On the other plate, locate the mannitol by first applying spray B, followed by spray C. The spots appearing white are mannitol. Measure the distance the solvent front traveled from the starting line and the distance the fluorescent spots are from the starting line. Calculate the R_f value by dividing the latter by the former. The mithramycin standard should have an R_f value of 0.7. If the standard has an R_f value greater than 0.8, the mobility of the standard may be decreased by increasing the ratio of the chloroform to methanol in the solvent to 3:2 or 3:1. Mithramycin appears as a single major component with the same R_f value as the mithramycin standard. It may show trace components at R_f values of about 0.5 and 0.4, and at the origin, which shall not be more intense than those shown by the mithramycin standard.

[19] Copies may be obtained from: American Statistical Association, 806 15th St. NW., Washington, DC 20005.

§ 450.245 Mitomycin for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Mitomycin for injection is a dry mixture of mitomycin and mannitol. Its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of mitomycin that it is represented to contain. It is sterile. It is nonpyrogenic. It passes the safety test. It contains no histamine nor histamine-like substances. Its moisture content is not more than 5 percent. Its pH, when reconstituted as directed in the labeling, is not less than 6.0 and not more than 8.0. It passes the identity test for mitomycin. The mitomycin used conforms to the standards prescribed by § 450.45 (a) (1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The mitomycin used in making the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(b) The batch for potency, sterility, pyrogens, safety, histamine, moisture, pH, and identity.

(ii) Samples required:

(a) The mitomycin used in making the batch: Five packages, each containing approximately 100 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 25 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents from each container if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the solution thus obtained with sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Further dilute the stock solution with solution 1 to the reference concentration of 1 microgram of mitomycin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 0.5 milligram of mitomycin per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this chapter, except observe the mice for 7 days in lieu of 48 hours, noting mortality every 24 hours. Repeat the test as described in § 436.33(d) of this chapter if one or more animals die within 7 days.

(5) *Histamine.* Proceed as directed in § 436.35 of this chapter.

(6) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(7) *pH.* Proceed as directed in § 436.202 of this chapter using the drug reconstituted as directed in the labeling.

(8) *Identity.* Proceed as directed in § 436.310 of this chapter.

PART 452—MACROLIDE ANTIBIOTIC DRUGS

Subpart A—Bulk Drugs

Sec.	
452.10	Erythromycin.
452.15	Erythromycin estolate.
452.20	Erythromycin ethylcarbonate.
452.25	Erythromycin ethylsuccinate.
452.25a	Sterile erythromycin ethylsuccinate.
452.30a	Sterile erythromycin gluceptate.
452.35	Erythromycin stearate.
452.40	Erythromycin sulfate.
452.75	Troleandomycin.

Subpart B—Oral Dosage Forms

452.110	Erythromycin oral dosage forms.
452.110a	Erythromycin tablets.
452.110b	Erythromycin enteric-coated tablets.
452.115	Erythromycin estolate oral dosage forms.
452.115a	Erythromycin estolate tablets.
452.115b	Erythromycin estolate capsules.
452.115c	Erythromycin estolate oral suspension.
452.115d	Erythromycin estolate for oral suspension.
452.115e	Erythromycin estolate for pediatric drops.
452.115f	Erythromycin estolate chewable tablets.
452.120	Erythromycin ethylcarbonate oral dosage forms.
452.120a	Erythromycin ethylcarbonate for oral suspension.

Sec.
452.120b Erythromycin ethylcarbonate for pediatric drops.
452.125 Erythromycin ethylsuccin te oral dosage forms.
452.125a Erythromycin ethylsuccinate tablets.
452.125b Erythromycin ethylsuccinate oral suspension.
452.125c Erythromycin ethylsuccinate for oral suspension.
452.135 Erythromycin stearate oral dosage forms.
452.135a Erythromycin stearate tablets.
452.135b Erythromycin stearate oral suspension.
452.175 Troleandomycin oral dosage forms.
452.175a Troleandomycin capsules.
452.175b Troleandomycin oral suspension.
452.175c Troleandomycin for oral suspension.
452.175d Troleandomycin chewable tablets.

Subpart C—Injectable Dosage Forms

452.225 Erythromycin ethylsuccinate injection.
452.232 Erythromycin lactobionate for injection.

Subpart D—Ophthalmic Dosage Forms

452.310 Erythromycin ophthalmic ointment.

Subpart F—Dermatologic Dosage Forms

452.510 Erythromycin dermatologic dosage forms.
452.510a Erythromycin ointment.
452.510b Erythromycin-neomycin sulfate ointment.

Subpart H—Rectal Dosage Forms

452.710 Erythromycin suppositories.

AUTHORITY: Sec. 507, 59 Stat. 463, as amended (21 U.S.C. 357).

Subpart A—Bulk Drugs

§ 452.10 Erythromycin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin is the odorless, white to grayish-white or slightly yellow compound of a kind of erythromycin or a mixture of two or more such compounds. It is so purified and dried that:

(i) It contains not less than 850 micrograms of erythromycin per milligram calculated on an anhydrous basis.

(ii) It passes the safety test.

(iii) Its moisture content is not more than 10 percent.

(iv) Its pH in a saturated aqueous solution, prepared by using 100 milligrams of erythromycin per milliliter, is not less than 8.0 or more than 10.5.

(v) Its residue on ignition is not more than 2.0 percent.

(vi) Its heavy metals content is not more than 50 parts per million.

(vii) It gives a positive identity test for erythromycin.

(viii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, moisture, residue on ignition, heavy metals, pH, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing not less than 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient methyl alcohol to give a concentration of 10 milligrams of erythromycin base per milliliter (estimated). Dilute this solution further with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution containing 1.0 milligram of erythromycin base per milliliter (estimated). Further dilute an aliquot of the stock solution with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Safety.* Proceed as directed in § 436.33 of this chapter.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a saturated aqueous solution (100 milligrams of erythromycin per milliliter).

(5) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(6) *Heavy metals.* Proceed as directed in § 436.208 of this chapter.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(8) *Identity test.* Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b)(3) of that section.

§ 452.15 Erythromycin estolate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin estolate is the lauryl sulfate salt of the propionyl ester of a kind of erythromycin or a mixture of two or more such salts. It occurs as a white powder. It is soluble in alcohol, methyl alcohol, acetone, and chloroform, but is practically insoluble in water. It is so purified and dried that:

(i) It contains not less than 600 micrograms of erythromycin per milligram, calculated on an anhydrous basis.

(ii) It passes the safety test.

(iii) Its moisture content is not more than 4.0 percent.

(iv) Its pH is not less than 4.5 nor more than 7.0.

(v) It gives positive identity tests for erythromycin estolate.

(vi) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, moisture, pH, identity, and crystallinity.

(ii) Samples of the batch: A minimum of 10 containers, each containing not less than 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient methyl alcohol to give a concentration of 1.0 milligram of erythromycin base per milliliter (estimated). Immediately dilute this solution further with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a concentration of 0.1 milligram of erythromycin per milliliter (estimated). Hydrolyze this solution in a 60° C. constant temperature water bath for 2 hours or at room temperature for 16 to 18 hours. Further dilute with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Safety.* Proceed as directed in § 436.33 of this chapter.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous suspension containing 100 milligrams per milliliter.

(5) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(6) *Identity test.* Proceed as directed in § 436.211 of this chapter, preparing the sample as described in pargraph (b)(3) of that section.

§ 452.20 Erythromycin ethylcarbonate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin ethylcarbonate is a white ester of erythromycin obtained by the reaction of a kind of erythromycin and chloroethylcarbonate. It is slightly soluble in water, freely soluble in alcohol, methyl alcohol, acetone, ether, and chloroform. It is so purified and dried that:

(i) It contains not less than 775 micrograms of erythromycin base per milligram, calculated on an anhydrous basis.

(ii) It passes the safety test.

(iii) Its moisture content is not more than 8 percent.

(iv) Its pH is not less than 6.3 and not more than 8.0.

(v) It gives a positive identity test for erythromycin ethylcarbonate.

(vi) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements prescribed by § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, moisture, pH, identity, and crystallinity.

(ii) Samples required: 10 containers, each containing not less than 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient methyl alcohol to give a concentration of 1.0 milligram of erythromycin base per milliliter (estimated). Immediately dilute this solution further with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a concentration of 0.1 milligram of erythromycin per milliliter (estimated). Place this solution in a 60° C. constant temperature water bath for 3 hours or

allow it to stand at room temperature for 24 to 40 hours to accomplish hydrolysis. Further dilute an aliquot with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Safety.* Proceed as directed in § 436.33 of this chapter.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a concentration of 200 milligrams of sample per milliliter.

(5) *Identity test.* Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b)(3) of that section.

(6) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

§ 452.25 Erythromycin ethylsuccinate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin ethylsuccinate is the white, odorless, ethylsuccinate ester of erythromycin. It is so purified and dried that:

(i) It contains not less than 765 micrograms of erythromycin per milligram, calculated on an anhydrous basis.

(ii) It passes the safety test.

(iii) Its moisture content is not more than 3.0 percent.

(iv) Its pH is not less than 6.0 and not more than 8.5.

(v) Its residue on ignition is not more than 1.0 percent.

(vi) It gives a positive identity test for erythromycin ethylsuccinate.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 (b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, moisture, pH, residue on ignition, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient methyl alcohol to give a concentration of 1 milligram of erythromycin base per milliliter (estimated). Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Safety.* Proceed as directed in § 436.33 of this chapter.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a 1.0 percent suspension in water.

(5) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using the sample prepared as described in paragraph (b)(3) of that section.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

§ 452.25a Sterile erythromycin ethylsuccinate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin ethylsuccinate is the white, odorless, ethylsuccinate ester of erythromycin. It is so purified and dried that:

(i) It contains not less than 765 micrograms of erythromycin per milligram, calculated on an anhydrous basis.

(ii) It is sterile.

(iii) It passes the safety test.

(iv) Its moisture content is not more than 3.0 percent.

(v) Its pH is not less than 6.0 and not more than 8.5.

(vi) Its residue on ignition is not more than 1.0 percent.

(vii) It gives a positive identity test for erythromycin ethylsuccinate.

(viii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, safety, moisture, pH, residue on ignition, identity, and crystallinity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 600 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient methyl alcohol to give a concentration of 1 milligram of erythromycin base per milliliter (estimated). Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(2) of that section.

(3) *Safety.* Proceed as directed in § 436.33 of this chapter.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using a 1.0 percent suspension in water.

(6) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(7) *Identity.* Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b)(3) of that section.

(8) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

§ 452.30a Sterile erythromycin gluceptate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin gluceptate is the white powder of the glucoheptonic acid salt of erythromycin or a mixture of two or more such salts. It is freely soluble in water, alcohol, and methyl alcohol. It is slightly soluble in acetone and chloroform, but is practically insoluble in ether. It is so purified and dried that:

(i) It contains not less than 600 micrograms of erythromycin per milligram, calculated on an anhydrous basis. If it is packaged for dispensing, its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of erythromycin that it is represented to contain.

(ii) It is sterile.

(iii) It passes the safety test.

(iv) It is nonpyrogenic.

(v) Its moisture content is not more than 5.0 percent.

(vi) Its pH in an aqueous solution containing 25 milligrams per milliliter is not less than 6.0 nor more than 8.0.

(vii) It gives a positive identity test for erythromycin gluceptate.

(2) *Packaging.* In addition to the requirements of § 432.1 of this chapter, if it is packaged for dispensing and intended for intravenous use, it shall contain the equivalent of 250 milligrams, 500 milligrams, or 1.0 gram of erythromycin per vial.

(3) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(4) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, safety, pyrogens, moisture, pH, and identity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use as an ingredient in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing not less than 300 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 12 immediate containers of the batch.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: If the batch is packaged for repacking or for use in manufacturing another drug, dissolve an accurately weighed sample in sufficient methyl alcohol to give a concentration of 10 milligrams of erythromycin base per milliliter (estimated). Dilute this solution further with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution containing 1.0 milligram of erythromycin base per milliliter (estimated). If it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypo-

dermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with solution 3 to give a stock solution of convenient concentration. Further dilute the stock solution with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 30 milligrams of erythromycin per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this chapter.

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using a concentration of 25 milligrams per milliliter.

(7) *Identity.* Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b)(2) of that section.

§ 452.35 Erythromycin stearate.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Erythromycin stearate is the odorless, white or slightly yellow powder of the stearic acid salt of erythromycin. It is practically insoluble in water but is soluble in alcohol, methyl alcohol, chloroform, and ether. It is so purified and dried that:

(i) It contains not less than 550 micrograms of erythromycin per milligram, calculated on an anhydrous basis.

(ii) It passes the safety test.

(iii) Its moisture content is not more than 4.0 percent.

(iv) Its pH is not less than 6.0 and not more than 11.0.

(v) Its residue on ignition is not more than 1.0 percent.

(vi) It gives positive identity tests for erythromycin stearate.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, moisture, pH, residue on ignition, identity, and crystallinity.

(ii) Samples required: A minimum of 10 containers, each consisting of 500 milligrams.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient methyl alcohol to give a concentration of 1 milligram of erythromycin base per milliliter (estimated). Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Safety.* Proceed as directed in § 436.33 of this chapter, preparing the sample as follows: Transfer approximately 1.0 to 1.1 grams to a mortar. Add 1 drop of 33 percent polysorbate 80 and while grinding with a pestle, slowly add sufficient sterile distilled water to make a suspension containing 80 milligrams of erythromycin base per milliliter.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a 1 percent slurry of erythromycin stearate in water.

(5) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b)(2) of that section.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

§ 452.40 Erythromycin sulfate.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Erythromycin sulfate is the sulfate salt of erythromycin. It is an odorless, practically white, powder. It is so purified and dried that:

(i) It contains not less than 840 micrograms of erythromycin per milligram, calculated on an anhydrous basis.

(ii) Its loss on drying is not more than 7.0 percent.

(iii) Its pH in a solution containing 10 milligrams per milliliter is not less than 4.5 and not more than 6.5.

(iv) It gives a positive identity test for erythromycin sulfate.

(v) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, crystallinity, and identity.

(ii) Samples required: 10 containers, each consisting of 300 milligrams.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient methyl alcohol to give a concentration of 1.0 milligram of erythromycin base per milliliter. Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of erythromycin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using a concentration of 10 milligrams per milliliter.

(4) *Identity.* Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b)(3) of that section.

§ 452.75 Troleandomycin.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Troleandomycin is the triacetyl ester of oleandomycin base or a mixture of two or more such esters. It is a white powder. It is so purified that:

(i) Its potency is not less than 750 micrograms of troleandomycin per milligram.

(ii) It passes the safety test.

(iii) Its loss on drying is not more than 1.0 percent.

(iv) Its pH in an aqueous alcohol solution containing 100 milligrams of troleandomycin per milliliter is not less than 7.0 and not more than 8.5.

(v) Its residue on ignition is not more than 0.1 percent.

(vi) It gives a positive identity test for oleandomycin.

(vii) Its R_f value by paper chromatography is approximately 0.85. If more than one spot appears on the paper chromatogram, determine its acetyl value, which is not less than 15.3 percent and not more than 16.0 percent.

(viii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, loss on drying, pH, residue on ignition, identity, R_f value, acetyl value (only if more than one spot is present in the determination of R_f value), and crystallinity.

(ii) Samples of the batch: 10 packages, nine containing approximately equal portions of not less than 500 milligrams, and one containing not less than 2.0 grams.

(b) *Tests and methods of assay—*(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(i) *Chemical method—*(*a*) *Reagents and equipment.* (*1*) Methyl orange reagent: Shake 0.5*M* boric acid solution for 12 hours (to ensure saturation) with an excess of methyl orange indicator. An alternative method is to heat the mixture to about 50° C. and shake for about an hour. Then allow to cool. Filter the saturated dye solution and wash three times with chloroform. Store the dye solution over chloroform.

(*2*) Acid-alcohol solution: Add 2 milliliters of concentrated sulfuric acid to 98 milliliters of absolute methyl alcohol.

(*3*) Glycerin: Reagent grade.

(*4*) Chloroform.

(*5*) Glacial acetic acid.

(*6*) Centrifuge tubes: 40 milliliters, glass-stoppered.

(*b*) *Procedure.* Using the troleandomycin working standard which has been dried for 3 hours at 60° C. and a pressure of 5 millimeters or less, prepare a standard solution in chloroform containing 50.0 milligrams of oleandomycin base in 200 milliliters. Transfer 10.0 milliliters of the solution to a 100-milliliter volumetric flask and dilute to volume with chloro-

form. Transfer 2.0, 4.0, 6.0, and 8.0 milliliters of this solution to glass-stoppered centrifuge tubes (40-milliliter size) and dilute to a total volume of 20.0 milliliters each with chloroform. To the 20 milliliters of the solution present in each 40-milliliter size centrifuge tube, add 0.2 milliliter of glacial acetic acid, 0.2 milliliter of glycerin, and 0.4 milliliter of methyl orange reagent. Shake for 5 minutes and centrifuge for 3 minutes. Immediately transfer to another tube a 10.0-milliliter aliquot from the chloroform (lower) layer. Care must be exercised to see that no portion of the dye-glycerin phase is included with the chloroform aliquot. Add 1.0 milliliter of acid-alcohol solution to this chloroform aliquot, mix well, and read the absorbancy at 535 nanometers, using a 1-centimeter cell and a suitable photometer and using chloroform, similiarly treated, as a blank. Prepare a standard curve, plotting the absorbance values of the standard solution against the concentration expressed in micrograms of oleandomycin base per aliquot. Accurately weigh the sample to be tested to give 50 milligrams (estimated) of oleandomycin base. Dissolve in chloroform and make to 200 milliliters with chloroform. Transfer 10.0 milliliters to a 100-milliliter volumetric flask and make to volume with chloroform. Transfer 5.0 milliliters to a glass-stoppered centrifuge tube and proceed as above. Determine the potency of the sample from the standard curve.

(ii) *Microbiological turbidimetric assay.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 80 percent isopropyl alcohol solution (solution 15) to give a stock solution containing 1,000 micrograms per milliliter. Further dilute the stock solution with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 25 micrograms of troleandomycin per milliliter (estimated).

(2) *Safety.* Proceed as directed in § 436.33 of this chapter.

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a saturated solution prepared by adding 100 milligrams of troleandomycin per milliliter of water-ethyl alcohol (1:1) diluent.

(5) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter, except use a silica crucible.

(6) *Identity.* Dissolve about 10 milligrams in 5 milliliters of hydrochloric acid and heat the solution in a boiling water bath; a greenish yellow color is produced.

(7) *Rf value*—(i) *Apparatus and reagents* (a) Chromatographic chamber (cylinder, glass-stoppered museum jar, 11.5 inches x 3.5 inches).

(b) Chromatographic paper (8 inches x 8 inches, Whatman No. 1).

(c) 0.1*N* hydrochloric acid.

(d) Resolving solvent: Butyl acetate, benzene, nitromethane, pyridine (5:5:5:1 by volume).

(e) Spray developing reagent: Place 1.0 milliliter of 10 percent platinic chloride solution and 25.0 milliliters of 4 percent potassium iodide solution in a 250-milliliter volumetric flask. Fill to mark with distilled water and mix well.

(ii) *Procedure.* Dissolve the sample in chloroform to give a solution containing 10 to 20 milligrams of oleandomycin base equivalent per milliliter. Prepare a sheet of chromatographic paper by drawing a line of origin parallel to and 1 inch from the edge of the paper. Wet the paper thoroughly with the 0.1*N* hydrochloric acid and blot it firmly between sheets of absorbent paper. Starting 2 inches in from the edge and at 1-inch intervals, apply 3 to 5 microliters of the sample solutions to the starting line. Allow a few minutes for the paper to dry partially. While it is still damp, form a cylinder by bringing the outer edges together, allowing about 1-inch overlap, and secure with a paper clip. Stand the paper in the chromatographic chamber, which has been filled to a depth of one-half of an inch with the resolving solvent. After the solvent front rises to a height of 4 to 5 inches above the origin, remove the paper from the tank and hang it up to air dry. Spray the dried paper with the developing reagent. Hang the paper in a 100° C. oven for 3 minutes. A purple spot becomes visible for troleandomycin at an R_f value of about 0.85. The approximate R_f values for diacetyloleandomycin, monoacetyloleandomycin, and oleandomycin are, respectively, 0.72, 0.27, and 0.13.

(8) *Acetyl determination*—(i) *Apparatus and reagents.* (a) One 3-necked Pyrex flask of approximately 45 milliliters capacity, pear-shaped with T-joints, agar inlet tube, glass-stoppered funnel, glass condenser, and bubble counter.

(b) 50-milliliter Pyrex Erlenmeyer flask.

(c) 10-milliliter buret, calibrated to 0.02 milliliter.

(d) Anhydrous methyl alcohol, reagent grade.

(e) 2*N* sodium hydroxide solution.

(f) Sulfuric acid solution prepared by adding 100 milliliters of concentrated H_2SO_4 to 200 milliliters of water.

(g) 1*N* barium chloride solution.

(h) Phenolphthalein solution (1 percent in ethyl alcohol).

(i) Water-pumped nitrogen.

(j) NaOH solution, 0.015*N*.

(ii) *Procedure.* Weigh accurately (to 0.01 milligram) approximately 30 milligrams of the sample into the three-necked acetyl flask. Add 2.0 milliliters of methyl alcohol to dissolve the sample; then add slowly, with gentle swirling, 1.0 milliliter of NaOH solution. Connect the gas inlet tube with bubble counter attached and adjust nitrogen flow to about two bubbles a second. Put glass-stoppered funnel in centerneck of acetyl flask and put about 5 milliliters of H_2O in the funnel. Add a boiling chip to the solution and attach condenser in the refluxing position with water cooling. Adjust burner flame under acetyl flask to reflux solution gently. Reflux for 30 minutes. Cool assembly slightly; then rinse down condenser (still in reflux position) with a few milliliters of H_2O. Reassemble condenser to the distillation position and add water through the funnel to make a total of approximately 5 milliliters of H_2O added to acetyl flask. Adjust burner flame so that about 5 milliliters of H_2O and methyl alcohol is distilled over in approximately 10 minutes. Discard this distillate. Cool acetyl flask slightly. Acidify solution in flask by adding 1 milliliter of the sulfuric acid solution through the funnel. Adjust burner flame and distill over approximately 20 milliliters of distillate into an Erlenmeyer flask in about 20 minutes, adding water through the funnel as necessary. It is important to keep the liquid volume in the acetyl flask around 2 to 3 milliliters in order to obtain a quantitative recovery of the acetic acid. Collect a second fraction of distillate, about 10 milliliters in volume. As the second fraction is distilling, process the first fraction. Heat the first fraction and boil gently about 20 seconds. Add a few drops of $BaCl_2$ solution to check if any sulfate was distilled over. If the sulfate is present, discard and repeat the whole determination. If the sulfate is absent, immediately titrate the solution with the 0.015*N* NaOH solution to a faint-pink endpoint, using one drop of phenolphthalein solution as the indicator. Repeat the above procedure with the second fraction. If the second fraction requires less than 0.10 milliliter of the 0.015*N* NaOH solution and all the acetic acid has been distilled over, the determination is completed. If greater than this, collect a third fraction of approximately 10 milliliters and titrate this as before. Total volumes of NaOH used and calculate results as follows:

$$\frac{\text{Milliliters of NaOH} \times N\text{NaOH} \times 0.043 \times 100}{\text{Weight sample in grams}} = \text{Percent acetyl.}$$

(9) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

Subpart B—Oral Dosage Forms

§ 452.110 Erythromycin oral dosage forms.

§ 452.110a Erythromycin tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin tablets are erythromycin with suitable and harmless buffer substances, diluents, binders, lubricants, colorings, flavorings, and suitable preservatives. The potency of each tablet is 75 milligrams, or 100 milligrams, or 250 milligrams of erythromycin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of erythromycin that it is represented to contain. Tablets shall disintegrate within 1 hour. The moisture content is not more than 7.5 percent. The erythromycin used in making the batch conforms to the standards prescribed by § 452.10(a)(1) (i), (ii), (iii), (iv), (v), (vii), and (viii).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin used in making the batch for potency, safety, pH, moisture, residue on ignition, crystallinity, and identity.

(*b*) The batch for potency, disintegration time, and moisture.

(ii) Samples required:

(*a*) The erythromycin used in making the batch: 10 packages, each containing 500 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Blend a representative number of tablets in a high-speed glass blender for 2 to 3 minutes with 200 milliliters of methyl alcohol. Add 300 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and blend again for 2 to 3 minutes. Further dilute with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter, using the procedure described in paragraph (e) (2) of that section.

§ 452.110b Erythromycin enteric-coated tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromyin enteric-coated tablets are enteric-coated tablets composed of erythromycin, suitable and harmless buffer substances, diluents, binders, lubricants, colorings, and flavorings. Each tablet contains 100 or 250 milligrams of erythromycin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of erythromycin that it is represented to contain. Each tablet shall meet the tests for enteric-coated tablets set forth in the U.S.P. and shall disintegrate within a total time of 2 hours. The moisture content is not more than 6 percent. The erythromycin base used in making the batch conforms to the standards of § 452.10(a) (1) (i), (ii), (iii), (iv), (v), (vii), and (viii).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin used in making the batch for potency, safety, moisture, pH, residue on ignition, crystallinity, and identity.

(*b*) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(*a*) The erythromycin used in making the batch: 10 packages, each containing 500 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Blend a representative number of tablets in a high-speed glass blender for 2 to 3 minutes with 200 milliliters of methyl alcohol. Add 300 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and blend again for 2 to 3 minutes. Further dilute with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter, using the procedure described in paragraph (e) (3) of that section.

§ 452.115 Erythromycin estolate oral dosage forms.

§ 452.115a Erythromycin estolate tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin estolate tablets are composed of erythromycin estolate with one or more suitable and harmless diluents, binders, lubricants, and colorings. Each tablet contains erythromycin estolate equivalent to 500 milligrams of erythromycin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of erythromycin that it is represented to contain. The moisture content is not more than 5 percent. The tablets shall disintegrate within 30 minutes. The erythromycin estolate used conforms to the standards prescribed by § 452.15(a) (1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin estolate used in making the batch for potency, safety, moisture, pH, identity, and crystallinity.

(*b*) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(*a*) The erythromycin estolate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch. A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of tables into a high-speed glass blender jar with 200 milliliters of methyl alcohol. Blend for 3 to 5 minutes. Add 300 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and blend again for 3 to 5 minutes. Hydrolyze a portion of this solution in a 60° C. constant temperature water bath for 2 hours or at room temperature for 16 to 18 hours. Further dilute with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter, using the procedure described in paragraph (e) (1) of that section.

§ 452.115b Erythromycin estolate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, quality, and purity.* Erythromycin estolate capsules are capsules containing erythromycin estolate with suitable and harmless buffer substances and diluents enclosed in a gelatin capsule. The erythromycin estolate content of each capsule is equivalent to either 250 milligrams of erythromycin or 125 milligrams of erythromycin. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of erythromycin that it is represented to contain. The moisture content is not more than 5 percent. The erythromycin estolate used conforms to the standards prescribed therefor by § 452.15(a) (1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain;

(i) Results of tests and assays on:

(*a*) The erythromycin estolate used in making the batch for potency, safety, pH, moisture, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The erythromycin estolate used in making the batch: 10 packages, each containing not less than 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Test and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Blend a representative number of capsules in a high-speed glass blender with 200 milliliters of methyl alcohol for 2 to 3 minutes. Add 300 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and blend again for 2 to 3 minutes. Hydrolyze a portion of this solution in a 60° C. constant temperature water bath for 2 hours or at room temperature for 16 to 18 hours. Further dilute with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 452.115c Erythromycin estolate oral suspensions.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin estolate oral suspension is erythromycin estolate with suitable and harmless buffer substances,

dispersing agents, diluents, coloring, and flavorings. Each milliliter contains erythromycin estolate equivalent to 25, 50, or 100 milligrams of erythromycin. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of erythromycin that it is represented to contain. Its pH is not less than 3.5 and not more than 6.5. The erythromycin estolate used conforms to the standards prescribed by § 452.15(a)(1).

(2) *Labeling.* In addition to conforming with the requirements of § 432.5 of this chapter, each package shall bear on its outside wrapper or container and the immediate container the statement "Refrigerate" or "Keep under refrigeration".

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin estolate used in making the batch for potency, safety, moisture, pH, crystallinity, and identity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The erythromycin estolate used in making the batch: 10 containers, each having not less than 300 milligrams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Remove an accurately measured representative volume of the suspension and dilute with sufficient methyl alcohol to give a concentration of 2.5 milligrams per milliliter (estimated). Dilute the entire mixture with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a concentration of 1.0 milligram of erythromycin base per milliliter (estimated). Hydrolyze in a 60° C. constant temperature water bath for 2 hours or at room temperature for 16 to 18 hours. Further dilute with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug as it is prepared for dispensing.

§ 452.115d Erythromycin estolate for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin estolate for oral suspension is a dry mixture of erythromycin estolate with suitable and harmless buffer substances, dispersing agents, diluents, colorings, and flavorings. The erythromycin estolate content is 25 milligrams of erythromycin per milliliter of the reconstituted suspension. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of erythromycin that it is represented to contain. When reconstituted as directed in its labeling, its pH is not less than 5.0 and not more than 7.0. Its moisture content is not more than 2.0 percent. The erythromycin estolate used conforms to the standards of § 452.15(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin estolate used in making the batch for potency, safety, moisture, pH, crystallinity, and identity.

(*b*) The batch: Potency, moisture, and pH.

(ii) Samples required:

(*a*) The erythromycin estolate used in making the batch: 10 immediate containers, each consisting of 300 milligrams.

(*b*) The batch: A minimum of 6 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Withdraw an accurately measured representative volume of the reconstituted suspension and add sufficient methyl alcohol to give a concentration of 2.5 milligrams of erythromycin base per milliliter (estimated). Dilute this entire mixture with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a concentration of 1.0 milligram of erythromycin base per milliliter (estimated). Hydrolyze in a 60° C. constant temperature water bath for 2 hours or at room temperature for 16 to 18 hours. Further dilute with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter, using the dry powder.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in its labeling.

§ 452.115e Erythromycin estolate for pediatric drops.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin estolate for pediatric drops is a dry mixture of erythromycin estolate with suitable and harmless dispersing agents, buffer substances, diluents, colorings, and flavorings. When reconstituted as directed in the labeling, each milliliter contains the equivalent of 100 milligrams of erythromycin. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of erythromycin that it is represented to contain. Its moisture content is not more than 2.0 percent. Its pH is not less than 5.0 nor more than 5.5. The erythromycin estolate used conforms to the standards prescribed by § 452.15(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin estolate used in making the batch for potency, safety, pH, moisture, crystallinity, and identity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The erythromycin estolate used in making the batch: 10 packages, each containing not less than 300 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Withdraw an accurately measured representative volume of the reconstituted suspension and add sufficient methyl alcohol to give a concentration of 2.5 milligrams of erythromycin base per milliliter (estimated). Dilute this entire mixture with sufficient 0.1*M* potassium phosphate buffer, pH 8 (solution 3), to give a concentration of 1.0 milligram of erythromycin base per milliliter (estimated). Hydrolyze in a 60° C. constant temperature water bath for 2 hours or at room temperature for 16 to 18 hours. Further dilute with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 (b) of this chapter, using the suspension prepared as directed in the labeling.

§ 452.115f Erythromycin estolate chewable tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin estolate chewable tablets are tablets composed of erythromycin estolate and suitable and harmless diluents, binders, buffers, colorings, and flavorings. Each tablet contains erythromycin estolate equivalent to either 125 or 250 milligrams of erythromycin. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of erythromycin that it is represented to contain. The moisture content is not more than 4 percent. The erythromycin estolate used in making the batch conforms to the standards prescribed by § 452.15(a)(1).

(2) *Labeling.* It shall be labeled in accordance with § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin estolate used in making the batch for potency, safety, moisture, pH, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The erythromycin estolate used in making the batch: 10 packages, each consisting of not less than 300 milligrams.

(b) The batch: A minimum of 30 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Blend a representative number of tablets in a high-speed glass blender for 2 to 3 minutes in 200 milliliters of methyl alcohol. Add 300 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and blend again for 2 to 3 minutes. Hydrolyze this solution in a 60° C. constant temperature water bath for 2 hours or at room temperature for 16 to 18 hours. Further dilute with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 452.120 Erythromycin ethylcarbonate oral dosage forms.

§ 452.120a Erythromycin ethylcarbonate for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin ethylcarbonate for oral suspension is a dry mixture of erythromycin ethylcarbonate with suitable and harmless buffer substances, dispersing agents, diluents, colorings, and flavorings. The erythromycin ethylcarbonate content is not less than 40 milligrams of erythromycin per milliliter of the reconstituted suspension. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of erythromycin that it is represented to contain. Its moisture content is not more than 2.0 percent. When reconstituted as directed in the labeling, its pH is not less than 6.4 and not more than 7.0. The erythromycin ethylcarbonate used conforms to the standards prescribed by § 452.20(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) Erythromycin ethylcarbonate used in making the batch for potency, safety, moisture, pH, crystallinity, and identity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The erythromycin ethylcarbonate used in making the batch: 10 containers, each containing 500 milligrams.

(*b*) The batch: A minimum of 6 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Withdraw an accurately measured representative volume of the reconstituted suspension and add sufficient methyl alcohol to give a concentration of 2.5 milligrams of erythromycin base per milliliter (estimated). Dilute this entire mixture with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a concentration of 1.0 milligram of erythromycin base per milliliter (estimated). Hydrolyze this solution by placing it in a 60° C. constant temperature water bath for 3 hours or by allowing it to stand at room temperature for 24 to 40 hours. Further dilute with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the suspension prepared as directed in its labeling.

§ 452.120b Erythromycin ethylcarbonate for pediatric drops.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin ethylcarbonate for pediatric drops is a dry mixture of erythromycin ethylcarbonate, suitable and harmless dispersing agents, buffer substances, diluents, colorings, and flavorings. When reconstituted as directed in the labeling, each milliliter contains 100 milligrams of erythromycin. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of erythromycin that it is represented to contain. Its moisture content is not more than 2.0 percent. Its pH is not less than 6.5 nor more than 7.5. The erythromycin ethylcarbonate used conforms to the standards prescribed by § 452.20(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin ethylcarbonate used in making the batch for potency, safety, pH, moisture, crystallinity, and identity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The erythromycin ethylcarbonate used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Withdraw an accurately measured representative volume of the reconstituted suspension and add sufficient methyl alcohol to give a concentration of 2.5 milligrams of erythromycin base per milliliter (estimated). Dilute this entire mixture with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a concentration of 1.0 milligram of erythromycin base per milliliter (estimated). Hydrolyze this solution by placing it in a 60° C. constant temperature water bath for 3 hours or by allowing it to stand at room temperature for 24 to 40 hours. Further dilute with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the suspension reconstituted as directed in the labeling.

§ 452.125 Erythromycin ethylsuccinate oral dosage forms.

§ 452.125a Erythromycin ethylsuccinate tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin ethylsuccinate tablets are composed of erythromycin ethylsuccinate, suitable and harmless diluents, binders, buffers, colorings, and flavorings. Each tablet contains erythromycin ethylsuccinate equivalent to 100 or 200 milligrams of erythromycin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of erythromycin that it is represented to contain. The moisture content is not more than 5 percent. The tablets shall disintegrate within 45 minutes. The erythromycin ethylsuccinate used conforms to the standards prescribed by § 452.25(a)(1).

(2) *Labeling.* It shall be labeled in accordance with § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin ethylsuccinate used in making the batch for potency, safety, moisture, pH, residue on ignition, identity, and crystallinity.

(*b*) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(*a*) The erythromycin ethylsuccinate used in making the batch: 10 packages, each consisting of 500 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Blend a representative number of tablets in a high-speed glass blender for 2 to 3 minutes with 200 milliliters of methyl alcohol. Add 300 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and blend again for 2 to 3 minutes. Further dilute with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter.

§ 452.125b Erythromycin ethylsuccinate oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin ethylsuccinate oral suspension is erythromycin ethylsuccinate with suitable and harmless buffer substances, dispersing agents, diluents, colorings, flavorings, and preservatives. Each milliliter con-

tains erythromycin ethylsuccinate equivalent to 40 or 80 milligrams of erythromycin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of erythromycin that it is represented to contain. Its pH is not less than 6.5 and not more than 8.5. The erythromycin ethylsuccinate used conforms to the standards prescribed by § 452.25 (a) (1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin ethylsuccinate used in making the batch for potency, safety, moisture, pH, identity, residue on ignition, and crystallinity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The erythromycin ethylsuccinate used in making the batch: 10 containers, each consisting of 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately measured representative volume of the suspension into a high-speed glass blender jar and add sufficient methyl alcohol to give a concentration of 1.0 milligram of erythromycin base per milliliter (estimated). Blend for 3 to 5 minutes. Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted drug.

§ 452.125c Erythromycin ethylsuccinate for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin ethylsuccinate for oral suspension is a dry mixture of erythromycin ethylsuccinate with suitable and harmless buffer substances, dispersing agents, diluents, colorings, and flavorings. It contains the equivalent of 40 milligrams of erythromycin per milliliter of the reconstituted suspension. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of erythromycin that it is represented to contain. Its loss on drying is not more than 1 percent. When reconstituted as directed in the labeling, its pH is not less than 7.0 nor more than 9.0. The crystalline erythromycin ethylsuccinate used conforms to the standards prescribed by § 452.25(a) (1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin ethylsuccinate used in making the batch for potency, safety, moisture, pH, residue on ignition, identity, and crystallinity.

(*b*) The batch, for potency, pH, and loss on drying.

(ii) Samples required:

(*a*) The erythromycin ethylsuccinate used in making the batch: 10 containers each consisting of approximately 500 milligrams.

(*b*) The batch: A minimum of 6 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Place an accurately measured representative volume of the reconstituted suspension into a 200-milliliter volumetric flask containing 100 milliliters of methyl alcohol and shake vigorously. Fill to volume with methyl alcohol and mix well. Allow to stand for about 5 minutes or until any undissolved particles settle. Dilute an aliquot of the supernatant liquid with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the suspension prepared as directed in the labeling.

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

§ 452.135 Erythromycin stearate oral dosage forms.

§ 452.135a Erythromycin stearate tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin stearate tablets are tablets composed of erythromycin stearate with suitable and harmless buffer substances, diluents, binders, lubricants, colorings, and flavorings. Each tablet contains erythromycin stearate equivalent to 75, 100, 125, 250, or 500 milligrams of erythromycin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of erythromycin that it is represented to contain. Tablets shall disintegrate within 1½ hours, except tablets containing sulfonamides shall disintegrate within 2½ hours. The moisture content is not more than 7 percent. The erythromycin stearate used in making the tablets conforms to the standards prescribed by § 452.35(a) (1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin stearate used in making the batch for potency, safety, pH, moisture, residue on ignition, crystallinity, and identity.

(*b*) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(*a*) The erythromycin stearate used in making the batch: 10 containers, each consisting of not less than 500 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Blend a representative number of tablets in a high-speed glass blender with 200 milliliters of methyl alcohol for 3 to 5 minutes. Add 300 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and blend again for 3 to 5 minutes. Further dilute with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter, using the procedure described in paragraph (e) (2) of that section.

§ 452.135b Erythromycin stearate oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin stearate oral suspension is erythromycin stearate with suitable and harmless buffer substances, dispersing agents, diluents, colorings, and flavorings. It contains the equivalent of 25 milligrams of erythromycin per milliliter. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of erythromycin that it is represented to contain. Its pH is not less than 7.0 and not more than 8.5. The erythromycin stearate used conforms to the standards prescribed by § 452.35(a) (1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin stearate used in making the batch for potency, safety, moisture, pH, residue on ignition, identity, and crystallinity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The erythromycin stearate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately measured representative volume of the suspension into a high-speed glass blender jar. Add sufficient methyl alcohol to the jar to give a concentration of 1.25 milligrams of erythromycin base per milliliter (estimated). Blend for 2 to 3 minutes. Add sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a concentration of 0.5 milligrams of erythromycin base per milliliter (esti-

mated) and blend again for 2 to 3 minutes. Further dilute with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted suspension.

§ 452.175 Troleandomycin oral dosage forms.

§ 452.175a Troleandomycin capsules.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Troleandomycin capsules are capsules composed of troleandomycin and one or more suitable buffers, diluents, binders, lubricants, and colorings. Each capsule contains 125 milligrams or 250 milligrams of troleandomycin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of troleandomycin that it is represented to contain. The loss on drying is not more than 5 percent. The troleandomycin used conforms to the standards prescribed by § 452.75(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The troleandomycin used in making the batch for potency, safety, loss on drying, pH, residue on ignition, identity, R_f value, acetyl value (only if more than one spot is present in the determination of R_f value), and crystallinity.

(*b*) The batch for potency and loss on drying.

(ii) Samples required:

(*a*) The troleandomycin used in making the batch: 10 packages, nine containing approximately equal portions of not less than 500 milligrams and one containing not less than 2 grams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules in a high-speed glass blender and add 500 milliliters of 80 percent isopropyl alcohol solution (solution 15). Blend for 3 minutes. Dilute an aliquot with sufficient solution 15 to give a stock solution containing 1,000 micrograms of troleandomycin per milliliter (estimated). Further dilute with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 25 micrograms of troleandomycin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

§ 452.175b Troleandomycin oral suspension.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Troleandomycin oral suspension is troleandomycin and one or more suitable buffers, dispersants, flavorings, colorings, and preservatives suspended in a suitable and harmless vehicle. Each milliliter contains 25 milligrams of troleandomycin. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of troleandomycin that it is represented to contain. Its pH is not less than 5.0 and not more than 8.0. The troleandomycin used conforms to the standards prescribed by § 452.75(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The troleandomycin used in making the batch for potency, safety, loss on drying, pH, residue on ignition, identity, R_f value, acetyl value (only if more than one spot is present in the determination of R_f value), and crystallinity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The troleandomycin used in making the batch: 10 packages, nine containing approximately equal portions of not less than 500 milligrams and one containing not less than 2 grams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dilute an appropriate sample (usually from 1.0 milliliter to 5.0 milliliters) with sufficient 80 percent isopropyl alcohol solution (solution 15) to give a stock solution containing 1,000 micrograms of troleandomycin per milliliter (estimated). Further dilute an aliquot with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 25 micrograms of troleandomycin per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted sample.

§ 452.175c Troleandomycin for oral suspension.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Troleandomycin for oral suspension is troleandomycin with suitable buffers, dispersants, preservatives, colorings, and flavorings. When the suspension is prepared as directed in its labeling, each milliliter contains 25 milligrams of troleandomycin. However, if it is for pediatric use, each milliliter contains 100 milligrams of troleandomycin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of troleandomycin that it is represented to contain. Its loss on drying is not more than 2 percent. The pH of the suspension, when prepared as directed in its labeling, is not less than 5.0 and not more than 7.0. The troleandomycin used conforms to the standards prescribed by § 452.75(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The troleandomycin used in making the batch for potency, safety, loss on drying, pH, residue on ignition, identity, R_f value, acetyl value (only if more than one spot is present in the determination of R_f value), and crystallinity.

(*b*) The batch for potency, loss on drying, and pH.

(ii) Samples required:

(*a*) The troleandomycin used in making the batch: 10 packages, nine containing approximately equal portions of not less than 500 milligrams and one containing not less than 2 grams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Reconstitute the drug as directed in the labeling. Dilute an appropriate sample (usually 1 milliliter to 5 milliliters) with sufficient 80 percent isopropyl alcohol solution (solution 15) to give a stock solution containing 1,000 micrograms of troleandomycin per milliliter (estimated). Further dilute an aliquot of the stock solution with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 25 micrograms of troleandomycin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the suspension obtained after reconstituting the drug as directed in its labeling.

§ 452.175d Troleandomycin chewable tablets.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Each troleandomycin chewable tablet contains an amount equivalent to 125 milligrams of oleandomycin with suitable diluents, binders, buffers, colorings, and flavorings. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of troleandomycin that it is represented to contain. The loss on drying is not more than 5 percent. The troleandomycin used conforms to the standards prescribed by § 452.75(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The troleandomycin used in making the batch for potency, safety, loss on drying, pH, residue on ignition, identity, R_f value, acetyl value (only if more than one spot is present in the determination of R_f value), and crystallinity.

(b) The batch for potency and loss on drying.

(ii) Samples required:

(a) The troleancomycin used in making the batch: 10 packages, nine containing approximately 500 milligrams each and one containing approximately 2 grams.

(b) The batch: A minimum of 30 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of tablets in a glass-blending jar with sufficient 80 percent isopropyl alcohol solution to give a stock solution of 1,000 micrograms of troleandomycin per milliliter (estimated). Blend 3 to 5 minutes. Remove an aliquot of the stock solution and dilute with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 25 micrograms of troleandomycin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

Subpart C—Injectable Dosage Forms

§ 452.225 Erythromycin ethylsuccinate injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin ethylsuccinate injection is erythromycin ethylsuccinate and butylaminobenzoate dissolved in polyethylene glycol 400. It contains a suitable and harmless preservative. Each milliliter contains 50 milligrams of erythromycin. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of erythromycin that it is represented to contain. It contains 2 percent butylaminobenzoate. It is sterile. It passes the safety test. Its moisture content is not more than 1.5 percent. The erythromycin ethylsuccinate used conforms to the standards prescribed therefore by § 452.25a(a)(1).

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5 of this chapter, each immediate container shall bear on its label and labeling the statement: "Warning—For intramuscular use only".

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The erythromycin ethylsuccinate used in making the batch for potency, moisture, pH, residue on ignition, identity, and crystallinity.

(b) The batch for potency, sterility, safety, and moisture.

(ii) Samples required:

(a) The erythromycin ethylsuccinate used in making the batch: 10 packages, each containing 500 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 10 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation, except that if the product is sterilized after filling, a representative sample consisting of 10 immediate containers from each sterilizer load. If only one sterilizer load is involved, the sample shall consist of 20 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: By means of a suitable hypodermic needle and syringe, remove an accurately measured representative volume of the sample and dilute with sufficient methyl alcohol to give a solution containing 1.0 milligram of erythromycin base per milliliter (estimated). Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use a bacterial-retentive membrane resistant to the solvent polyethylene glycol 400 and add 1 milliliter from each immediate container directly to the membrane, thus eliminating the preliminary solubilization step.

(3) *Safety.* Proceed as directed in § 436.33 of this chapter, except in lieu of paragraph (b) of that section, administer subcutaneously a test dose of 0.1 milliliter of the undiluted solution.

(4) *Moisture.* Proceed as directed in § 436.201(e)(1) of this chapter.

§ 452.232 Erythromycin lactobionate for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin lactobionate for injection is a dry mixture of erythromycin lactobionate and a suitable preservative. It contains the equivalent of 300 milligrams, 500 milligrams, or 1 gram of erythromycin per vial. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of erythromycin that it is represented to contain. It is sterile. It is nonpyrogenic. It passes the safety test. Its moisture content is not more than 5 percent. Its pH is not less than 6.5 and not more than 7.5. The erythromycin used conforms to the standards prescribed by § 452.10(a)(1)(i), (iii), (iv), (v), (vi), (vii), and (viii).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The erythromycin used in making the batch for potency, pH, moisture, residue on ignition, heavy metals, and crystallinity.

(b) The batch for potency, sterility, pyrogens, safety, moisture, pH, and identity.

(ii) Samples required:

(a) The erythromycin used in making the batch: 10 containers, each consisting of not less than 500 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 12 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Using a suitable hypodermic needle and syringe, remove the total withdrawable contents from each container represented as a single-dose container; or if the labeling specifies the amount of potency in a given volume of the preparation, withdraw an accurately measured volume from each container. Dilute with sterile distilled water to obtain a concentration of 10 milligrams of erythromycin base per milliliter (estimated). Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 30 milligrams of erythromycin per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this chapter.

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using a concentration of 50 milligrams of erythromycin per milliliter.

(7) *Identity.* Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b)(2) of that section.

Subpart D—Ophthalmic Dosage Forms

§ 452.310 Erythromycin ophthalmic ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin ophthalmic ointment is erythromycin in a suitable and harmless ointment base. Each gram of ointment contains 5 milligrams of erythromycin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of erythromycin that it is represented to contain. It is sterile. The moisture content is not more than 1 percent. The erythromycin used conforms to the standards prescribed by § 452.10(a)(1)(i), (ii), (iii), (iv), (v), (vii), and (viii).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The erythromycin used in making the batch for potency, safety, pH, moisture, residue on ignition, crystallinity, and identity.

(b) The batch for potency, sterility, and moisture.

(ii) Samples required:

(a) The erythromycin used in making the batch: 10 packages, each containing 500 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of five immediate containers.

(2) For sterility testing: Twenty immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the ointment in a 250-milliliter separatory funnel containing 50 milliliters of reagent-grade petroleum ether. Shake until dissolved. Wash with four separate washings of a 4:1 mixture of methyl alcohol and distilled water. Combine the washings and bring to volume with the methyl alcohol-water solution in a volumetric flask. Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(3) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

Subpart F—Dermatologic Dosage Forms

§ 452.510 Erythromycin dermatologic dosage forms.

§ 452.510a Erythromycin ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin ointment is erythromycin in a suitable and harmless ointment base. It may contain suitable preservatives. Each gram of ointment contains 10 milligrams of erythromycin. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of erythromycin that it is represented to contain. The moisture content is not more than 1.0 percent. The erythromycin used conforms to the standards prescribed by § 452.10(a)(1) (i), (iii), (iv), (v), (vii), and (viii).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The erythromycin used in making the batch for potency, pH, moisture, residue on ignition, crystallinity, and identity.

(b) The batch for potency and moisture.

(ii) Samples required:

(a) The erythromycin used in making the batch: 10 packages, each containing not less than 500 milligrams.

(b) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the ointment in a 250-milliliter separatory funnel containing 50 milliliters of reagent-grade petroleum ether. Shake until dissolved. Wash with four separate washings of a 4:1 mixture of methyl alcohol and distilled water. Combine the washings and bring to volume with the methyl alcohol-water solution in a volumetric flask. Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 452.510b Erythromycin-neomycin sulfate ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin-neomycin sulfate ointment is erythromycin and neomycin sulfate with a suitable emollient and perservatives in a suitable and harmless ointment base. Each gram contains 5 milligrams of erythromycin and 3.5 milligrams of neomycin. Its erythromycin content is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of erythromycin that it is represented to contain. Its neomycin content is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of neomycin that it is represented to contain. The moisture content is not more than 1 percent. The erythromycin used conforms to the standards prescribed therefor by § 452.10(a)(1) (i), (iii), (iv), (v), (vii), and (viii). The neomycin sulfate used conforms to the standards prescribed in § 444.42a(a)(1) (i), (vi), (vii), and (viii) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The erythromycin used in making the batch for potency, pH, moisture, residue on ignition, crystallinity, and identity.

(b) The neomycin sulfate used in making the batch for potency, pH, loss on drying, and identity.

(c) The batch for erythromycin content, neomycin content, and moisture.

(ii) Samples required:

(a) The erythromycin used in making the batch: 10 packages, each containing not less than 500 milligrams.

(b) The neomycin sulfate used in making the batch: 10 packages, each containing not less than 300 milligrams.

(c) The batch: A minimum of 6 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows:

(i) *Erythromycin content.* Place an accurately weighed representative portion of the ointment in a high-speed glass blender with 100 milliliters of polyethylene glycol 400 and blend for 3 minutes. Filter through a cotton plug or filter paper and further dilute with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(ii) *Neomycin content.* Place an accurately weighed representative portion of the ointment in a separatory funnel containing 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction with new portions of the buffer at least three times and any additional times necessary to ensure complete extraction of the antibiotic. Combine the buffer extractives and adjust to an appropriate volume with solution 3 to give a stock solution of convenient concentration. Place the stock solution in a second separatory funnel and wash with three 30-milliliter portions of peroxide-free ether. Discard the ether washes. Further dilute the washed stock solution with solution 3 to the reference concentration of 1.0 microgram of neomycin base per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

Subpart H—Rectal Dosage Forms

§ 452.710 Erythromycin suppositories.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin suppositories contain in each suppository 125 milligrams of erythromycin in a suitable and harmless base. The erythromycin content is satisfactory if it is not less than 90 percent nor more than 120 percent of the number of milligrams of erythromycin that it is represented to contain. The moisture content is not more than 1.0 percent. The erythromycin used conforms to the standards prescribed by § 452.10(a)(1), (i), (ii), (iii), (iv), (v) (vii), and (viii), except its moisture content is not more than 5.0 percent.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The erythromycin used in making the batch for potency, safety, moisture, pH, residue on ignition, identity, and crystallinity.

(b) The batch for potency and moisture.

(ii) Samples required:

(a) The erythromycin used in making the batch: 10 packages, each containing not less than 500 milligrams.

(b) The batch: A minimum of 30 suppositories.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Blend a representative number of suppositories for 3 to 5 minutes in a high-speed glass blender with 200 milliliters of methyl alcohol. Add 300 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and blend again for 3 to 5 minutes. Remove an aliquot and dilute with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

PART 453—LINCOMYCIN ANTIBIOTIC DRUGS

Subpart A—Bulk Drugs

Sec.
453.20 Clindamycin hydrochloride hydrate.
453.21 Clindamycin palmitate hydrochloride.
453.22a Sterile clindamycin phosphate.
453.30 Lincomycin hydrochloride monohydrate.
453.30a Sterile lincomycin hydrochloride.

Subpart B—Oral Dosage Forms

453.120 Clindamycin hydrochloride hydrate capsules.
453.121 Clindamycin palmitate hydrochloride oral dosage forms.
453.121a Clindamycin palmitate hydrochloride for oral suspension.
453.121b Clindamycin palmitate hydrochloride for oral solution.
453.130 Lincomycin hydrochloride oral dosage forms.
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Subpart C—Injectable Dosage Forms

453.222 Clindamycin phosphate injection.
453.230 Lincomycin hydrochloride injection.

AUTHORITY: Sec. 507, 59 Stat. 463, as amended (21 U.S.C. 357).

Subpart A—Bulk Drugs

§ 453.20 Clindamycin hydrochloride hydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Clindamycin hydrochloride hydrate is the hydrated hydrochloride salt of clindamycin. It is so purified and dried that:

(i) Its clindamycin content is not less than 800 micrograms of clindamycin per milligram.

(ii) Its microbiological activity is not less than 800 micrograms of clindamycin per milligram.

(iii) It passes the safety test.

(iv) Its moisture content is not less than 3.0 percent and not more than 6.0 percent.

(v) Its pH in an aqueous solution containing 100 milligrams per milliliter is not less than 3.0 and not more than 5.5.

(vi) It is crystalline.

(vii) It passes the identity test for clindamycin hydrochloride hydrate.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for clindamycin content, microbiological activity, safety, moisture, pH, crystallinity, and identity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Clindamycin content (vapor phase chromatography).* Proceed as directed in § 436.302 of this chapter.

(2) *Microbiological activity (microbiological agar diffusion assay.)* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to give a stock solution of convenient concentration. Further dilute the stock solution with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of clindamycin per milliliter (estimated).

(3) *Safety.* Proceed as directed in § 436.33 of this chapter.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams per milliliter.

(6) *Crystallinity.* Proceed as directed in § 436.203 of this chapter.

(7) *Identity.* Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b) (2) of that section.

§ 453.21 Clindamycin palmitate hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Clindamycin palmitate hydrochloride is the white to off-white amorphous powder of the hydrochloride salt of the palmitic acid ester of clindamycin. It is freely soluble in water, ethanol, chloroform, and ether. It is so purified and dried that:

(i) It contains not less than 540 micrograms of clindamycin per milligram.

(ii) It passes the safety test.

(iii) Its moisture content is not more than 3.0 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.8 and not more than 3.8.

(v) It passes the identity test for clindamycin palmitate hydrochloride.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for clindamycin content, safety, moisture, pH, and identity.

(ii) Samples required: 10 packages, nine containing not less than 300 milligrams and one package containing not less than 2 grams.

(b) *Tests and methods of assay*—(1) *Clindamycin content.* Proceed as directed in § 436.303 of this chapter.

(2) *Safety.* Proceed as directed in § 436.33 of this chapter.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Identity.* Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b) (2) of that section.

§ 453.22a Sterile clindamycin phosphate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile clindamycin phosphate is a water-soluble ester of clindamycin and phosphoric acid. It occurs as a white to off-white powder. It is so purified and dried that:

(i) Its clindamycin content is not less than 758 micrograms of clindamycin per milligram calculated on an anhydrous basis.

(ii) Its microbiological activity is not less than 758 micrograms of clindamycin per milligram calculated on an anhydrous basis.

(iii) It is sterile.

(iv) It is nonpyrogenic.

(v) It passes the safety test.

(vi) It contains no histamine nor histamine-like substances.

(vii) Its moisture content is not more than 6 percent.

(viii) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 3.5 and not more than 4.5.

(ix) It is crystalline.

(x) It passes the identity test for clindamycin phosphate.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for clindamycin content, microbiological activity, sterility, pyrogens, safety, histamine, moisture, pH, crystallinity, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, nine containing approximately 300 milligrams and one containing 1.5 grams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Clindamycin content (vapor phase chromatography).* Proceed as directed in § 436.304 of this chapter.

(2) *Microbiological activity (microbiological agar diffusion assay).* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Accurately weigh approximately 12 milligrams of the clindamycin phosphate sample into a 50-milliliter glass-stoppered centrifuge tube. Pipet 25 milliliters of the pH 9.0 borate buffer into the cen-

trifuge tube. Add 10 milliliters of chloroform and shake vigorously for 15 minutes. Centrifuge the resulting mixture and pipet a 20-milliliter aliquot of the aqueous phase into a 35-milliliter centrifuge tube. Add a weighed amount of intestinal alkaline phosphatase equivalent to 50 units of activity[1] and allow the solution to stand until the enzyme has completely dissolved. Place the tube into a water bath at 37° C.±2° C. for 2.5 hours. After the 2.5-hour hydrolysis, allow the solution to cool. Further dilute an aliquot of the solution with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of clindamycin per milliliter (estimated).

(3) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(4) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 24 milligrams of clindamycin per milliliter.

(5) *Safety.* Proceed as directed in § 436.33 of this chapter.

(6) *Histamine.* Proceed as directed in § 436.35 of this chapter.

(7) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(8) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(9) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(10) *Identity.* Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b)(2) of that section, except dry the sample for 2 hours at 100° C. and allow to equilibrate with the atmosphere for 1 hour.

§ 453.30 Lincomycin hydrochloride monohydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Lincomycin hydrochloride monohydrate is the monohydrated hydrochloride salt of lincomycin. It is freely soluble in water and soluble in acetone and dimethylformamide. It is so purified and dried that:

(i) Its potency is not less than 790 micrograms of lincomycin per milligram.

(ii) It passes the safety test.

(iii) Its moisture content is not less than 3.0 percent and is not more than 6.0 percent.

(iv) Its pH in an aqueous solution containing 100 milligrams per milliliter is not less than 3.0 and not more than 5.5.

(v) Its specific rotation in an aqueous solution at 25° C. is not less than +135° and not more than +150°.

(vi) It passes the infrared identity test.

(vii) Its content of lincomycin B is not more than 5 percent.

(viii) It passes the identity test if the elution pattern of the lincomycin sample compares quantitatively to that of the lincomycin working standard under identical conditions of vapor phase chromatography.

(ix) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, moisture, pH, specific rotation, infrared absorption spectrum, lincomycin B content, crystallinity, and identity.

(ii) Samples of the batch: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to give a stock solution of convenient concentration. Further dilute the stock solution with 0.1*M* potassium phosphate buffer, pH 8 (solution 3), to the reference concentration of 2 micrograms of lincomycin per milliliter (estimated).

(ii) *Vapor phase chromatography assay.* Proceed as directed in § 436.306 of this chapter.

(2) *Safety.* Proceed as directed in § 436.33 of this chapter.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams per milliliter.

(5) *Specific rotation.* Accurately weigh 500 milligrams of lincomycin hydrochloride monohydrate in a 25-milliliter, glass stoppered volumetric flask and fill to volume with distilled water. Proceed as directed in § 436.210 of this chapter, using a 2.0-decimeter polarimeter tube and calculate the specific rotation on an anhydrous basis.

(6) *Infrared absorption spectrum.* Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b)(2) of that section.

(7) *Lincomycin B content.* Proceed as directed in § 436.306 of this chapter.

(8) *Identity.* Proceed as described in § 436.306 of this chapter.

(9) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

§ 453.30a Sterile lincomycin hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Lincomycin hydrochloride monohydrate is the monohydrated hydrochloride salt of lincomycin. It is freely soluble in water and soluble in acetone and dimethylformamide. It is so purified and dried that:

(i) Its potency is not less than 790 micrograms of lincomycin per milligram.

(ii) It is sterile.

(iii) It passes the safety test.

(iv) It is nonpyrogenic.

(v) It contains no histamine nor histamine-like substances.

(vi) Its moisture content is not less 3.0 percent and not more than 6.0 percent.

(vii) Its pH in an aqueous solution containing 100 milligrams per milliliter is not less than 3.0 and not more than 5.5.

(viii) Its specific rotation in an aqueous solution at 25° C. is not less than +135° and not more than +150°.

(ix) It passes the infrared identity test.

(x) Its content of lincomycin B is not more than 5 percent.

(xi) It passes the identity test if the elution pattern of the lincomycin sample compares quantitatively to that of the lincomycin working standard under identical conditions of vapor phase chromatography.

(xii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, safety, pyrogens, histamine, moisture, pH, specific rotation, infrared absorption spectrum, lincomycin B content, identity, and crystallinity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to give a stock solution of convenient concentration. Further dilute the stock solution with 0.1*M* potassium phosphate buffer, pH 8 (solution 3), to the reference concentration of 2 micrograms of lincomycin per milliliter (estimated).

(ii) *Vapor phase chromatography assay.* Proceed as directed in § 436.306 of this chapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Safety.* Proceed as directed in § 436.33 of this chapter.

(4) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 0.5 milligram of lincomycin per milliliter.

[1] Defined such that 50 units hydrolyzes at least 20 micromoles of a clindamycin phosphate authentic sample under the assay conditions described in this section.

(5) *Histamine.* Proceed as directed in § 436.35 of this chapter.

(6) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(7) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams per milliliter.

(8) *Specific rotation.* Accurately weigh 500 milligrams of lincomycin hydrochloride monohydrate in a 25 milliliter, glass-stoppered volumetric flask and fill to lincomycin B content, crystallinity, and volume with distilled water. Proceed as directed in § 436.210, using a 2.0-decimeter polarimeter tube and calculate the specific rotation on an anhydrous basis.

(9) *Infrared absorption spectrum.* Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b) (2) of that section.

(10) *Lincomycin B content.* Proceed as directed in § 436.306 of this chapter.

(11) *Identity.* Proceed as directed in § 436.306 of this chapter.

(12) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

Subpart B—Oral Dosage Forms

§ 453.120 Clindamycin hydrochloride hydrate capsules.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Clindamycin hydrochloride hydrate capsules are composed of clindamycin hydrochloride hydrate and one or more suitable and harmless diluents and lubricants. Each capsule contains clindamycin hydrochloride hydrate equivalent to 75 or 150 milligrams of clindamycin. Its content of clindamycin is satisfactory if it is not less than 90 percent and not more than 120 percent of the amount of clindamycin that it is represented to contain. The moisture content is not more than 7.0 percent. The clindamycin hydrochloride hydrate used conforms to the standards prescribed by § 453.20(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The clindamycin hydrochloride hydrate used in making the batch for clindamycin content, microbiological activity, safety, moisture, pH, crystallinity, and identity.

(*b*) The batch for clindamycin content and moisture.

(ii) Samples required:

(*a*) The clindamycin hydrochloride hydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay—*(1) *Clindamycin content (vapor phase chromatography).* Proceed as directed in § 436.302 of this chapter, except:

(i) *Preparation of clindamycin sample and working standard solutions.* Accurately weigh a portion of the clindamycin working standard equivalent to about 45 milligrams of clindamycin and transfer to a 15-milliliter glass-stoppered centrifuge tube. Empty 20 capsules, collecting the contents quantitatively. Weigh the powder and determine the average capsule fill weight. Mix the powder and accurately weigh a portion containing the equivalent of about 45 milligrams of clindamycin into a second 15-milliliter glass-stoppered centrifuge tube. Add 3 milliliters of 1 percent sodium carbonate solution and 3 milliliters of chloroform to each tube. Shake the solution vigorously and then centrifuge. Remove the top aqueous layer and add approximately 1 gram of anhydrous sodium sulfate to dry the chloroform layer. Place a 1-milliliter aliquot of the chloroform solution into a 15-milliliter centrifuge tube, add 1 milliliter of internal standard and 0.6 milliliter of acetic anhydride. Agitate the vials to insure complete mixing of the liquids.

(ii) *Calculations.* Calculate the clindamycin content of the capsules as follows:

$$\text{Milligrams of clindamycin per capsule} = \frac{R_u \times W_s \times f \times W_c}{R_s \times W_u}$$

where:

R_u = Area of the clindamycin sample peak (at a retention time equal to that observed for the clindamycin standard) / Area of internal standard peak;

R_s = Area of the clindamycin standard peak / Area of internal standard peak;

W_s = Weight of clindamycin working standard in milligrams;

W_u = Sample weight in milligrams;

f = Potency of clindamycin working standard in milligrams of clindamycin per milligram;

W_c = Average capsule fill weight in milligrams.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 453.121 Clindamycin palmitate hydrochloride oral dosage forms.

§ 453.121a Clindamycin palmitate hydrochloride for oral suspension.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Clindamycin palmitate hydrochloride for oral suspension is composed of clindamycin palmitate hydrochloride with one or more suitable and harmless diluents, buffer substances, colorings, and flavorings. When reconstituted as directed in the labeling, using the accompanying diluent when provided, each milliliter contains clindamycin palmitate hydrochloride equivalent to 15 milligrams of clindamycin. Its clindamycin content is satisfactory if it is not less than 90 percent and not more than 120 percent of the amount of clindamycin that it is represented to contain. The moisture content is not more than 3.0 percent. When reconstituted as directed in the labeling, its pH is not less than 3.0 and not more than 5.0. The clindamycin palmitate hydrochloride used conforms to the standards prescribed by § 453.21(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The clindamycin palmitate hydrochloride used in making the batch for clindamycin content, safety, moisture, pH, and identity.

(*b*) The batch for clindamycin content, moisture, and pH.

(ii) Samples required:

(*a*) The clindamycin palmitate hydrochloride used in making the batch: 10 packages, nine containing not less than 300 milligrams, and one containing not less than 2 grams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay—*(1) *Clindamycin content.* Proceed as directed in § 436.303 of this chapter, except:

(i) Preparation of clindamycin palmitate hydrochloride sample and working standard solutions. Accurately weigh about 130 milligrams of the clindamycin palmitate hydrochloride working standard and transfer to a 25-milliliter volumetric flask. Add 5 milliliters of distilled water. Reconstitute the clindamycin palmitate hydrochloride for oral suspension as directed in the labeling, using the accompanying diluent when provided, and transfer exactly 5.0 milliliters to a 25-milliliter volumetric flask. Add exactly 5.0 milliliters of internal standard and 1 milliliter of 30 percent sodium carbonate to each flask. Shake both flasks mechanically for 5 minutes. Transfer the contents of each flask to separate 15-milliliter glass-stoppered centrifuge tubes and centrifuge. Remove the top aqueous layer by suction and transfer exactly 1.0 milliliter of the chloroform layer to separate glass-stoppered, conical, 15-milliliter centrifuge tubes. Add 1 milliliter of pyridine and 0.5 milliliter of acetic anhydride. Agitate the tubes to insure complete mixing of the liquids. Proceed as directed in § 436.303(a) of this chapter.

(ii) Calculations: Calculate the clindamycin content as follows:

$$\text{Milligrams of clindamycin per milliliter} = \frac{R_u \times W_s \times f}{R_s \times V},$$

where:

R_u = Area of the sample peak (at a retention time equal to that observed for the clindamycin palmitate hydrochloride standard) / Area of internal standard peak

R_s = Area of the clindamycin palmitate hydrochloride standard peak / Area of internal standard peak

W_s = Weight of the clindamycin palmitate hydrochloride working standard in milligrams;
V = Volume of reconstituted sample in milliliters;
f = Milligrams of clindamycin activity per milligram of clindamycin palmitate hydrochloride working standard.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) pH. Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

§453.121b Clindamycin palmitate hydrochloride for oral solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Clindamycin palmitate hydrochloride for oral solution is composed of clindamycin palmitate hydrochloride with one or more suitable and harmless diluents, buffer substances, colorings, flavorings, and preservatives. When reconstituted as directed in the labeling, each milliliter contains clindamycin palmitate hydrochloride equivalent to 15 milligrams of clindamycin. Its clindamycin content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of clindamycin that it is represented to contain. The moisture content is not more than 3.0 percent. When reconstituted as directed in the labeling, its pH is not less than 2.5 and not more than 5.0. The clindamycin palmitate hydrochloride used conforms to the standards prescribed by § 453.21(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The clindamycin palmitate hydrochloride used in making the batch for clindamycin content, safety, moisture, pH, and identity.

(*b*) The batch for clindamycin content, moisture, and pH.

(ii) Samples required:

(*a*) The clindamycin palmitate hydrochloride used in making the batch: 10 packages, nine containing not less than 300 milligrams, and one containing not less than 2 grams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Clindamycin content.* Proceed as directed in § 436.303 of this chapter, except:

(i) *Preparation of clindamycin palmitate hydrochloride sample and working standard solutions.* Accurately weigh about 130 milligrams of the clindamycin palmitate hydrochloride working standard and transfer to a 25-milliliter volumetric flask. Add 5 milliliters of distilled water. Reconstitute the clindamycin palmitate hydrochloride for oral solution as directed in the labeling and transfer exactly 5.0 milliliters to a 25-milliliter volumetric flask. Add exactly 5.0 milliliters of internal standard and 1 milliliter of 30-percent sodium carbonate to each flask. Shake both flasks mechanically for 5 minutes. Transfer the contents of each flask to separate 15-milliliter glass-stoppered centrifuge tubes and centrifuge. Remove the top aqueous layer by suction and transfer exactly 1.0 milliliter of the chloroform layer to separate glass-stoppered, conical, 15-milliliter centrifuge tubes. Add 1 milliliter of pyridine and 0.5 milliliter of acetic anhydride. Agitate the tubes to insure complete mixing of the liquids. Proceed as directed in § 436.303(e) of this subchapter.

(ii) *Calculations.* Calculate the clindamycin content as follows:

$$\text{Milligrams of clindamycin per milliliter} = \frac{R_u \times W_s \times f}{R_s \times V}$$

where:

R_u = Area of the sample peak (at a retention time equal to that observed for the clindamycin palmitate hydrochloride standard); / Area of internal standard peak

R_s = Area of the clindamycin palmitate hydrochloride standard peak; / Area of internal standard peak

W_s = Weight of the clindamycin palmitate hydrochloride working standard in milligrams;
V = Volume of reconstituted sample in milliliters;
f = Milligrams of clindamycin activity per milligram of clindamycin palmitate hydrochloride working standard

(2) *Moisture.* Proceed as directed in § 436.201 of this subchapter.

(3) *pH.* Proceed as directed in § 436.202 of this subchapter, using the drug reconstituted as directed in the labeling.

§ 453.130 Lincomycin hydrochloride oral dosage forms.

§ 453.130a Lincomycin hydrochloride monohydrate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Lincomycin hydrochloride monohydrate capsules are composed of lincomycin hydrochloride monohydrate and suitable diluents, enclosed in a gelatin capsule. Each capsule contains 250 milligrams of lincomycin or 500 milligrams of lincomycin. The lincomycin content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of lincomycin that it is represented to contain. Its moisture content is not more than 7.0 percent. The lincomycin hydrochloride monohydrate used conforms to the standards prescribed by § 453.30(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The lincomycin hydrochloride monohydrate used in making the batch for potency, safety, moisture, pH, specific rotation, infrared absorption spectrum, identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The lincomycin hydrochloride monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules in a high-speed glass blender with sufficient 0.1*M* potassium phosphate buffer, pH 8 (solution 3), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 3 to the reference concentration of 2 micrograms of lincomycin per milliliter (estimated).

(ii) *Vapor phase chromatography assay.* Proceed as directed in § 436.306 of this chapter, except prepare the sample for assay as follows: Place the contents of 5 capsules in a 100-milliliter volumetric flask and add about 60 milliliters of methanol. Place on a steam bath and allow to boil gently for 5 minutes. Remove from the steam bath, add more methanol, and adjust to mark after cooling to ambient temperature. Dilute an aliquot equivalent to 50 milligrams of lincomycin to 25 milliliters with methanol. Transfer 2 milliliters to a centrifuge tube and evaporate to dryness on a steam bath with a stream of dry air. Dissolve the residue in 1 milliliter of dry pyridine. Calculate the lincomycin content of the capsules as follows:

$$\text{Lincomycin content in milligrams per capsule} = \frac{(Ru)(Ws)(d)(f)}{(Rs)(N)},$$

where:

$$Ru = \frac{\text{Area of lincomycin sample peak}}{\text{Area of internal standard}};$$

$$Rs = \frac{\text{Area of lincomycin standard peak}}{\text{Area of internal standard}};$$

Ws = Weight of lincomycin working standard in milligrams;
d = Dilution factor;
f = Potency of lincomycin working standard in milligrams of lincomycin per milligram;
N = Number of capsules used.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 453.130b Lincomycin hydrochloride sirup.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Lincomycin hydrochloride sirup is a sirup containing lincomycin hydrochloride monohydrate, one or more suitable preservatives, flavorings, sweetening agents, colorings, and purified water. Each milliliter contains lincomycin hydrochloride equivalent to either 25 milligrams or 50 milligrams of lincomycin. Its lincomycin content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of lincomycin that it is represented to contain. The pH is not less than 3 and not more than 6.5. The lincomycin hydrochloride monohydrate used conforms to the standards prescribed by § 453.30(a) (1) (i), (ii), (iv), (v), (vi), (vii), (viii), and (ix).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The lincomycin hydrochloride monohydrate used in making the batch for potency, safety, pH, specific rotation, infrared absorption spectrum, lincomycin B content, crystallinity, and identity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The lincomycin hydrochloride monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay—*(1) *Potency.* Use either of the following methods; however, the results obtained from the method described in paragraph (b) (1) (i) of this section shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Remove an accurately measured sample of the sirup with a suitable syringe. Place into a high-speed glass blender with sufficient 0.1*M* potassium phosphate buffer, pH 8 (solution 3), to give a total volume of 500 milliliters. Blend 3 to 5 minutes. Further dilute with solution 3 to the reference concentration of 2 micrograms of lincomycin per milliliter (estimated).

(ii) *Vapor phase chromatography assay.* Proceed as directed in § 436.306 of this chapter, except prepare the sample for assay by either of the following methods:

(*a*) Place an aliquot of sirup, containing the equivalent of 250 milligrams of lincomycin into a 50-milliliter volumetric flask and add 30 milliliters of absolute ethanol. Place on a steam bath and boil gently for 5 minutes. Remove from the steam bath and cool. Add ethanol to prior volume level and let stand overnight. Adjust to mark, shake well, and transfer a 5-milliliter aliquot into a 25-milliliter volumetric flask and make to mark with methanol. Place 4 milliliters of this solution in a 15-milliliter centrifuge tube and evaporate to dryness on a steam bath with a stream of dry air. Dissolve the residue in 1 milliliter of dry pyridine. Calculate the lincomycin content as follows:

$$\text{Lincomycin content in milligrams per milliliter} = \frac{(Ru)(Ws)(d)(f)}{(Rs)(M)},$$

where:

$$Ru = \frac{\text{Area of lincomycin sample peak}}{\text{Area of internal standard}};$$

$$Rs = \frac{\text{Area of lincomycin standard peak}}{\text{Area of internal standard}};$$

Ws = Weight of lincomycin working standard in milligrams;
d = Dilution factor;
f = Potency of lincomycin working standard in milligrams of lincomycin per milligram;
M = Milliliters of sirup used.

(*b*) Treat the lincomycin working standard and sample in a similar manner, except lyophilize an aliquot of the sample containing the equivalent of 50 milligrams of lincomycin. To approximately 50 milligrams of the standard, accurately weighed, and to the dried residue of the sample, add 5 milliliters of dry pyridine which contains 10 milligrams of tetraphenylcyclopentadienone per milliliter. Warm on a hot plate for 5 minutes to attain complete solution. Remove from the hot plate and add 5 milliliters of hexamethyldisilazane and 2 milliliters of trimethylchlorosilane. Shake mechanically for 60 minutes, then centrifuge for 15 minutes. Inject 2 microliters of the supernate into the chromatograph. Calculate the lincomycin content as follows:

$$\text{Lincomycin content in milligrams per milliliter of sirup} = \frac{(Ru)(Ws)(f)}{(Rs)(M)},$$

where:

$$Ru = \frac{\text{Area of lincomycin sample peak}}{\text{Area of internal standard}};$$

$$Rs = \frac{\text{Area of lincomycin standard peak}}{\text{Area of internal standard}};$$

Ws = Weight of lincomycin working standard in milligrams;
f = Potency of lincomycin working standard in milligrams of lincomycin per milligram;
M = Milliliters of sirup used.

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted sample.

Subpart C—Injectable Dosage Forms

§ 453.222 Clindamycin phosphate injection.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Clindamycin phosphate injection is an aqueous solution of clindamycin phosphate with one or more suitable and harmless preservatives and sequestering agents. Each milliliter contains clindamycin phosphate equivalent to 150 milligrams of clindamycin. Its clindamycin content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of clindamycin that it is represented to contain. It is sterile. It is nonpyrogenic. It passes the safety test.

It contains no histamine nor histamine-like substances. Its pH is not less than 5.5 and not more than 7. The clindamycin phosphate used conforms to the standards prescribed by § 453.22a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The clindamycin phosphate used in making the batch for clindamycin content, microbiological activity, moisture, pH, crystallinity, and identity.

(*b*) The batch for clindamycin content, sterility, pyrogens, safety, histamine, and pH.

(ii) Samples required:

(*a*) The clindamycin phosphate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Clindamycin content (vapor phase chromatography).* Proceed as directed in § 436.304 of this chapter, except prepare the sample for assay as follows: Shake the sample and dilute a portion with pH 9.0 borate buffer to obtain a solution containing the equivalent of approximately 0.4 milligram of clindamycin per milliliter. Place 25 milliliters of this solution into a 50-milliliter stoppered centrifuge tube. Add 10 milliliters of chloroform. Shake vigorously for 15 minutes and centrifuge. There should be no emulsion present after centrifugation. Transfer 20 milliliters of the aqueous phase from the tube into a 35-milliliter stoppered centrifuge tube. Add to the tube a weighed amount of intestinal alkaline phosphatase equivalent to 50 units of activity [12] and allow to stand until the phosphatase has dissolved completely. Place the centrifuge tube into a water bath at 37° C.±2° C. for 2.5 hours. After the 2.5-hour hydrolysis, allow the solution to cool.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing the equivalent of 24 milligrams of clindamycin per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this chapter.

(5) *Histamine.* Proceed as directed in § 436.35 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted drug.

[12] Defined such that 50 units hydrolyzes at least 20 micromoles of a clindamycin phosphate authentic sample under the assay conditions described in § 436.304 of this chapter.

§ 453.230 Lincomycin hydrochloride injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Lincomycin hydrochloride injection is an aqueous solution of lincomycin hydrochloride monohydrate containing benzyl alcohol as a preservative. Each immediate container contains either 1, 2, or 10 milliliters of a solution containing, in each milliliter, 300 milligrams of lincomycin, and 9 milligrams of benzyl alcohol. The lincomycin content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of lincomycin that it is represented to contain. It is sterile. It passes the safety test. It is nonpyrogenic. It contains no histamine or histamine-like substances. Its pH is not less than 3.0 and not more than 5.5. The lincomycin hydrochloride monohydrate used conforms to the standards prescribed by § 453.30a(a)(1)(i), (vi), (vii), (viii), (ix), (x), and (xi).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. If each immediate container contains only 1 milliliter of the drug, the labeling shall include the statement "For pediatric use".

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The lincomycin hydrochloride monohydrate used in making the batch for potency, moisture, pH, specific rotation, infrared absorption spectrum, lincomycin B content, identity, and crystallinity.

(*b*) The batch for potency, sterility, safety, pyrogens, histamine, and pH.

(ii) Samples required:

(*a*) The lincomycin hydrochloride monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Place the portion into a suitable volumetric flask and bring to mark with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3). Remove an aliquot and dilute with solution 3 to the reference concentration of 2.0 micrograms of lincomycin per milliliter (estimated).

(ii) *Vapor phase chromatography assay.* Proceed as directed in § 436.306 of this chapter, except prepare the sample for assay as follows: Dilute the equivalent of 300 milligrams of lincomycin to 50 milliliters with methanol and shake. Transfer a 3-milliliter aliquot to a 10-milliliter volumetric flask and make to mark with methanol. Place a 2-milliliter aliquot into a 15-milliliter centrifuge tube and evaporate to dryness on a steam bath with a stream of dry air. Dissolve the residue in 1 milliliter of dry pyridine. Calculate the lincomycin content as follows:

$$\text{Lincomycin content in milligrams per milliliter} = \frac{(Ru)(Ws)(d)(f)}{(Rs)(\text{number of milliliters of sample})}$$

where:

$$Ru = \frac{\text{Area of lincomycin sample peak}}{\text{Area of internal standard}};$$

$$Rs = \frac{\text{Area of lincomycin standard peak}}{\text{Area of internal standard}};$$

Ws = Weight of lincomycin working standard in milligrams;

d = Dilution factor;

f = Potency of lincomycin working standard in milligrams of lincomycin per milligram.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Safety.* Proceed as directed in § 436.33 of this chapter.

(4) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 0.5 milligram of lincomycin per milliliter.

(5) *Histamine.* Proceed as directed in § 436.35 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted solution.

PART 455—CERTAIN OTHER ANTIBIOTIC DRUGS

Subpart A—Bulk Drugs

Sec.	
455.3	Calcium amphomycin.
455.10	Chloramphenicol.
455.10a	Sterile chloramphenicol.
455.10b	Sterile chloramphenicol.
455.11	Chloramphenicol palmitate.
455.12a	Sterile chloramphenicol sodium succinate.
455.20	Cycloserine.
455.50	Calcium novobiocin.
455.51	Sodium novobiocin.
455.51a	Sterile sodium novobiocin.
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Sec.
455.80a Sterile spectinomycin hydrochloride.
455.85 Vancomycin hydrochloride.
455.85a Sterile vancomycin hydrochloride.

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455.110 Chloramphenicol capsules.
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455.150 Calcium novobiocin oral suspension.
455.151 Sodium novobiocin oral dosage forms.
455.151a Sodium novobiocin tablets.
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455.310 Chloramphenicol ophthalmic dosage forms.
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455.310d Chloramphenicol-polymyxin ointment.

Subpart E—Otic Dosage Forms

455.410 Chloramphenicol otic; chloramphenicol topical.

Subpart F—Dermatologic Dosage Forms

455.508a Calcium amphomycin-neomycin sulfate-hydrocortisone acetate cream.
455.510 Chloramphenicol dermatologic dosage forms.
455.510a Chloramphenicol ointment (chloramphenicol cream).
455.510b Chloramphenicol otic; chloramphenicol topical.
455.510c Chloramphenicol-polymyxin ointment.

AUTHORITY: Sec. 507, 59 Stat. 463, as amended (21 U.S.C. 357).

Subpart A—Bulk Drugs

§ 455.3 Calcium amphomycin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Calcium amphomycin is the calcium salt of amphomycin. It is so purified and dried that:

(i) Its potency is not less than 868 micrograms of amphomycin per milligram.

(ii) Its moisture content is not more than 10 percent.

(iii) Its pH in a 2 percent aqueous suspension is not less than 6.0 and not more than 7.5.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, and pH.

(ii) Samples required on the batch: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Allow this stock solution to stand overnight at room temperature to assure that the sample is dissolved completely. Further dilute the stock solution with solution 3 to the reference concentration of 10.0 micrograms of amphomycin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using a suspension prepared by adding 20 milligrams per milliliter.

§ 455.10 Chloramphenicol.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chloramphenicol is a white to grayish-white or yellowish-white powder, occurring as needles or elongated plates. It is neutral, slightly soluble in water, but freely soluble in alcohol. It has the chemical formula D-(—)-*threo*-1-*p*-nitrophenyl-2 - dichloracetamido-1,3-propanediol. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram.

(ii) It passes the safety test.

(iii) Its pH in a saturated aqueous solution is not less than 4.5 nor more than 7.5.

(iv) Its specific rotation in absolute ethyl alcohol at 20° C. is +20°±1.5°, and at 25° C. is +18.5°±1.5°.

(v) Its melting range is 151°±2° C.

(vi) Its absorptivity at 278 nanometers is ±3 percent of that of the chloramphenicol working standard similarly treated.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, pH, specific rotation, melting range, absorptivity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(i) *Microbiological turbidimetric assay.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient ethyl alcohol to give a solution containing 10,000 micrograms of chloramphenicol per milliliter (estimated). Add sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a concentration of 1,000 micrograms of chloramphenicol per milliliter (estimated). Further dilute an aliquot with solution 1 to the reference concentration of 2.5 micrograms of chloramphenicol per milliliter (estimated).

(ii) *Spectrophotometric method.* Dissolve approximately 50 milligrams each of the sample and working standard in 100 milliliters of distilled water. Warm if necessary to hasten dissolution. Transfer 10 milliliters into a 250-milliliter volumetric flask and fill to volume with distilled water. Using a suitable spectrophotometer equipped with a 1-centimeter cell and distilled water as the blank, determine the absorbance of each solution at 278 nanometers. Calculate the potency of chloramphenicol as follows:

$$\text{Potency of sample in micrograms per milligram} = \frac{\text{Absorbance of sample} \times \text{weight of standard in milligrams} \times \text{potency of standard in micrograms per milligram}}{\text{Absorbance of standard} \times \text{weight of sample in milligrams}}$$

(2) *Safety.* Proceed as directed in § 436.33 of this chapter. Apply sufficient heat to dissolve the chloramphenicol.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using a saturated aqueous solution.

(4) *Specific rotation.* Accurately weigh approximately 1.25 grams of the sample into a 25-milliliter glass-stoppered volumetric flask and dissolve in about 15 milliliters of absolute alcohol, warming if necessary. Dilute the solution to 25 milliliters with absolute alcohol and mix thoroughly. Proceed as directed in § 436.210 of this chapter, using a 2.0-decimeter polarimeter tube.

(5) *Melting range.* Proceed as directed in § 436.209 of this chapter.

(6) *Absorptivity.* Proceed as directed in paragraph (b)(1)(ii) of this section, except calculate the percent relative absorptivity as follows:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{weight of standard in milligrams} \times \text{potency of standard in micrograms per milligram}}{\text{Absorbance of standard} \times \text{weight of sample in milligrams} \times 10}$$

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

§ 455.10a Sterile chloramphenicol.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile chloramphenicol is a white to grayish-white or yellowish-white powder, occurring as needles or elongated plates. It is neutral, slightly soluble in water, but freely soluble in alcohol. It has the chemical for-

mula D-(—)-*threo*-1-*p*-nitrophenyl-2-dichloracetamido-1,3-propanediol. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It passes the safety test.

(v) It contains no histamine nor histamine-like substances.

(vi) Its pH in a saturated aqueous solution is not less than 4.5 nor more than 7.5.

(vii) Its specific rotation in absolute ethyl alcohol at 20° C. is +20°±1.5°, and at 25° C. is +18.5°±1.5°.

(viii) Its melting range is 151°±2° C.

(ix) Its absorptivity at 278 nanometers is ±3 percent of that of the chloramphenicol working standard similarly treated.

(x) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, safety, histamine, pH, specific rotation, melting range, absorptivity, and crystallinity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 50 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(i) *Microbiological turbidimetric assay.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient ethyl alcohol to give a solution containing 10,000 micrograms of chloramphenicol per milliliter (estimated). Add sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a concentration of 1,000 micrograms of chloramphenicol per milliliter (estimated). Further dilute an aliquot with solution 1 to the reference concentration of 2.5 micrograms of chloramphenicol per milliliter (estimated).

(ii) *Spectrophotometric method.* Dissolve approximately 50 milligrams each of the sample and working standard in 100 milliliters of distilled water. Warm if necessary to hasten dissolution. Transfer 10 milliliters into a 250-milliliter volumetric flask and fill to volume with distilled water. Using a suitable spectrophotometer equipped with a 1-centimeter cell and distilled water as the blank, determine the absorbance of each solution at 278 nanometers. Calculate the potency of chloramphenicol as follows:

$$\text{Potency of sample in micrograms per milligram} = \frac{\text{Absorbance of sample} \times \text{weight of standard in milligrams} \times \text{potency of standard in micrograms per milligram}}{\text{Absorbance of standard} \times \text{weight of sample in milligrams}}$$

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use 50 milligrams in lieu of 300 milligrams.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 5 milligrams of chloramphenicol per milliliter. Apply sufficient heat to dissolve the chloramphenicol.

(4) *Safety.* Proceed as directed in § 436.33 of this chapter. Apply sufficient heat to dissolve the chloramphenicol.

(5) *Histamine.* Proceed as directed in § 436.35 of this chapter. Apply sufficient heat to dissolve the chloramphenicol.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using a saturated aqueous solution.

(7) *Specific rotation.* Accurately weigh approximately 1.25 grams of the sample in a 25-milliliter glass-stoppered volumetric flask and dissolve in about 15 milliliters of absolute alcohol, warming if necessary. Dilute the solution to 25 milliliters with absolute alcohol and mix thoroughly. Proceed as directed in § 436.210 of this chapter, using a 2.0 decimeter polarimeter tube.

(8) *Melting range.* Proceed as directed in § 436.209 of this chapter.

(9) *Absorptivity.* Proceed as directed in paragraph (b)(1)(ii) of this section except calculate the percent relative absorptivity as follows:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{weight of standard in milligrams} \times \text{potency of standard in micrograms per milligram}}{\text{Absorbance of standard} \times \text{weight of sample in milligrams} \times 10}$$

(10) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

§ 455.10b Sterile chloramphenicol.

(a) *Requirements for certification.* (1) *Standards of identity, strength, quality, and purity.* Chloramphenicol is a white to grayish-white or yellowish-white crystalline powder, occurring as needles or elongated plates. It is neutral, slightly soluble in water, but freely soluble in alcohol. It has the chemical formula D-(—)-*threo*-1-*p*-nitrophenyl-2-dichloracetamido-1,3-propanediol. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram;

(ii) It is sterile;

(iii) It is nontoxic;

(iv) It is nonpyrogenic;

(v) It contains no histamine nor histamine-like substances;

(vi) Its pH in saturated aqueous solution is not less than 4.5 and not more than 7.5;

(vii) Its specific rotation in absolute ethanol at 20° C. is +20°±1.5°, and at 25° C. is +18.5° ±1.5°;

(viii) Its melting point is 151° C. ±2°;

(ix) Its absorptivity at 278 mμ does not vary more than ±3 percent from that of the chloramphenicol working standard, similarly treated.

(2) *Packaging.* It shall be packaged in accordance with the requirements of § 432.1 of this chapter.

(3) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, safety, histamine, pH, specific rotation, melting range, absorptivity, and crystallinity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 50 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(i) *Microbiological turbidimetric assay.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient ethyl alcohol to give a solution containing 10,000 micrograms of chloramphenicol per milliliter (estimated). Add sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a concentration of 1,000 micrograms of chloramphenicol per milliliter (estimated). Further dilute an aliquot with solution 1 to the reference concentration of 2.5 micrograms of chloramphenicol per milliliter (estimated).

(ii) *Spectrophotometric method.* Dissolve approximately 50 milligrams each of the sample and working standard in 100 milliliters of distilled water. Warm if necessary to hasten dissolution. Transfer 10 milliliters into a 250-milliliter volumetric flask and fill to volume with distilled water. Using a suitable spectrophotometer equipped with a 1-centimeter cell and distilled water as the blank, determine the absorbance of each solution at 278 nanometers. Calculate the potency of chloramphenicol as follows:

$$\text{Potency of sample in micrograms per milligram} = \frac{\text{Absorbance of sample} \times \text{weight of standard in milligrams} \times \text{potency of standard in micrograms per milligram}}{\text{Absorbance of standard} \times \text{weight of sample in milligrams}}$$

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use 50 milligrams in lieu of 300 milligrams.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 5 milligrams of chloramphenicol per milliliter. Apply sufficient heat to dissolve the chloramphenicol.

(4) *Safety.* Proceed as directed in § 436.33 of this chapter. Apply sufficient heat to dissolve the chloramphenicol.

(5) *Histamine.* Proceed as directed in § 436.35 of this chapter. Apply sufficient heat to dissolve the chloramphenicol.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using a saturated aqueous solution.

(7) *Specific rotation.* Accurately weigh approximately 1.25 grams of the sample in a 25-milliliter glass-stoppered volumetric flask and dissolve in about 15 milliliters of absolute alcohol, warming if necessary. Dilute the solution to 25 milliliters with absolute alcohol and mix thoroughly. Proceed as directed in § 436.210 of this chapter, using a 2.0 decimeter polarimeter tube.

(8) *Melting range.* Proceed as directed in § 436.209 of this chapter.

(9) *Absorptivity.* Proceed as directed in paragraph (b)(1)(ii) of this section except calculate the percent relative absorptivity as follows:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{weight of standard in milligrams} \times \text{potency of standard in micrograms per milligram}}{\text{Absorbance of standard} \times \text{weight of sample in milligrams} \times 10}$$

(10) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

§ 455.11 Chloramphenicol palmitate.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Chloramphenicol palmitate is the white to grayish-white, tasteless palmitic acid ester of chloramphenicol. It is so purified and dried that:

(i) It contains not less than 555 micrograms nor more than 595 micrograms of chloramphenicol per milligram.

(ii) It passes the safety test.

(iii) Its melting range is 91°±4° C.

(iv) Its specific rotation in absolute ethyl alcohol at 25° C. is +23°±2°.

(v) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, melting range, specific rotation, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay—*(1) *Potency.* Dissolve approximately 90 milligrams of the sample and 50 milligrams of the chloramphenicol working standard in separate 100-milliliter volumetric flasks with absolute ethyl alcohol. Fill to volume with absolute ethyl alcohol. Transfer 10.0 milliliters of each solution into separate 250-milliliter volumetric flasks and fill to volume with absolute ethyl alcohol. Using a suitable spectrophotometer equipped with a 1.0-centimeter cell and absolute ethyl alcohol as a blank, determine the absorbance of the sample at 271 nanometers and that of the chloramphenicol working standard at 276 nanometers. Calculate the potency of the chloramphenicol palmitate as follows:

$$\text{Potency of sample in micrograms per milligram} = \frac{\text{Absorbance of sample} \times \text{weight of standard in milligrams} \times \text{potency of standard in micrograms per milligram}}{\text{Absorbance of standard} \times \text{weight of sample in milligrams}}$$

(2) *Safety.* Proceed as directed in § 436.33 of this chapter.

(3) *Melting range.* Proceed as directed in § 436.209 of this chapter.

(4) *Specific rotation.* Accurately weigh approximately 1.25 grams of sample in a 25-milliliter, glass-stoppered volumetric flask and dissolve in about 15 milliliters of absolute alcohol, warming if necessary to effect solution. Bring the solution to 25° C. Dilute the solution to 25 milliliters with absolute alcohol and mix thoroughly. Proceed as directed in § 436.210 of this chapter, using a 2.0-decimeter polarimeter tube.

(5) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

§ 455.12a Sterile chloramphenicol sodium succinate.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Chloramphenicol sodium succinate is the light-yellow, water-soluble, ethanol-soluble sodium salt of the 3-monosuccinate ester of chloramphenicol. It is so purified and dried that:

(i) Its potency is not less than 650 and not more than 765 micrograms per milligram. If it is packaged for dispensing, its potency when reconstituted as directed in the labeling is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of chloramphenicol per milliliter that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It passes the safety test.

(v) It contains no histamine nor histamine-like substances.

(vi) Its moisture content is not more than 5.0 percent.

(vii) Its pH in an aqueous solution containing 250 milligrams of chloramphenicol per milliliter is not less than 6.4 and not more than 7.0.

(viii) Its specific rotation in an aqueous solution containing 50 milligrams per milliliter at 25° C. is +6.5°±1.5°.

(ix) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterilty, pyrogens, safety, histamine, moisture, pH, specific rotation, and crystallinity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 500 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 8 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay—*(1) *Potency—*(i) *Working standard.* Dissolve an accurately weighed portion of the chloramphenicol working standard in sufficient distilled water to give a solution containing 20 micrograms per milliliter. Using a suitable spectrophotometer and distilled water as the blank, determine the absorbance of this solution in a 1-centimeter cell at a wavelength of 278 nanometers.

(ii) *Procedure.* Dissolve an accurately weighed portion of the sample to be tested in sufficient distilled water to give a solution containing 30 micrograms of the sample per milliliter (estimated); and also if it is packaged for dispensing, reconstitute as directed in the labeling. Remove an accurately measured representative portion from each container and further dilute this portion with sufficient distilled water to give a concentration of 20 micrograms of chloramphenicol per milliliter (estimated). Using a suitable spectrophotometer and distilled water as the blank, determine the absorbance of this solution in a 1-centimeter cell at a wavelength of 276 nanometers. Calculate the micrograms per milligram of the dry powder as follows:

$$\text{Micrograms of chloramphenicol per milligram} = \frac{\text{Absorbance of sample at 276 nanometers} \times \text{micrograms of standard per milliliter} \times \text{potency of chloramphenicol working standard in micrograms per milligram}}{\text{Absorbance of standard at 278 nanometers} \times \text{micrograms of sample per milliliter}}$$

Calculate the milligrams per milliliter of the reconstituted solution in the dispensing container as follows:

$$\text{Milligrams per milliliter of the reconstituted vial} = \frac{\text{Absorbance of sample at 276 nanometers} \times \text{micrograms of standard per milliliter} \times \text{labeled content of reconstituted vial in milligrams per milliliter}}{\text{Absorbance of standard at 278 nanometers} \times 20}$$

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 5 milligrams of chloramphenicol per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this chapter.

(5) *Histamine.* Proceed as directed in § 436.35 of this chapter.

(6) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(7) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 250 milligrams of chloramphenicol per milliliter.

(8) *Specific rotation.* Dilute the sample with sufficient distilled water to give a solution containing approximately 50 milligrams per milliliter. Proceed as directed in § 436.210 of this chapter, using a 1.0-decimeter polarimeter tube.

(9) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

§ 455.20 Cycloserine.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Cycloserine is a white to slightly yellowish compound. It has the chemical structure D-4-amino-3-isoxazolidone. It is so purified that:

(i) Its potency is not less than 900 micrograms per milligram.

(ii) It passes the safety test.

(iii) Its loss on drying is not more than 1.0 percent.

(iv) Its pH in a 10 percent aqueous solution is not less than 5.5 and not more than 6.5.

(v) Its residue on ignition is not more than 0.5 percent.

(vi) It gives a positive identity for cycloserine.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, loss on drying, pH, residue on ignition, crystallinity, and identity.

(ii) Samples of the batch: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay—*(1) *Potency—*Using the cycloserine working standard as the standard of comparison, assay for potency by either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(i) *Colorimetric assay—(a) Stock standard solution.* Dry approximately 100 milligrams of the working standard for 3 hours at 60° C. and a pressure of 5 millimeters or less. Determine the dry weight and dissolve the dried working standard in sufficient distilled water to give a solution containing 1,000 micrograms per milliliter. This solution may be used for 1 month if kept under refrigeration.

(b) *Standard curve solutions.* Pipette accurately 0.0, 1.0, 5.0, 10.0, 15.0, and 20.0 milliliters of the stock standard solution to each of six 100-milliliter volumetric flasks, dilute to 100 milliliters with 0.1*N* sodium hydroxide and mix thoroughly.

(c) Reagents:

(1) Acetic acid—1.0*N* solution.

(2) Sodium hydroxide—4.0*N* and 0.1*N* solutions.

(3) Sodium nitroprusside—4.0 percent solution: Dissolve 4.0 grams in sufficient distilled water to make 100.0 milliliters. Mix well. Store in amber bottle.

(4) Oxidized nitroprusside reagent—Mix equal parts of 4 percent sodium nitroprusside solution and 4.0*N* sodium hydroxide, and let stand for 1 hour before using. Prepare daily, and store in an amber bottle.

(d) *Procedure.* Transfer approximately 100 milligrams of sample, accurately weighed, to a 100 milliliter volumetric flask. Dissolve in sufficient 0.1*N* sodium hydroxide to measure exactly 100 milliliters. Mix thoroughly and transfer 10 milliliters to a second 100-milliliter volumetric flask, and mix thoroughly. Transfer exactly 1.0 milliliter of each of the standard curve solutions and of the sample solution to respective test tubes. Add exactly 3.0 milliliters of 1.0*N* acetic acid to each of the test tubes. Mix thoroughly. Add exactly 1.0 milliliter of oxidized nitroprusside reagent to each test tube and mix thoroughly. Allow the tubes to stand at room temperature for at least 10 minutes in order that maximum color intensity may develop. Using the solution containing 0.0 milliliter of working standard as a blank, determine the absorbances of the solutions at 625 nanometers in a suitable spectrophotometer. Plot concentration versus absorbance on linear graph paper. The curve may deviate slightly from a straight line. The standard curve solutions equal 0, 10, 50, 100, 150, and 200 micrograms of cycloserine, respectively.

(e) Calculations:

$$\text{Micrograms cycloserine per milligram} = \frac{\text{Concentration in micrograms from calibration curve} \times 1{,}000}{\text{Weight of original sample in milligrams.}}$$

(ii) *Microbiological turbidimetric assay.* Proceed as described in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to give a stock solution of convenient concentration. Further dilute the stock solution with sterile distilled water to the reference concentration of 50 micrograms of cycloserine per milliliter (estimated).

(2) *Safety.* Proceed as directed in § 436.33 of this chapter.

NOTE: Solution must be administered within ½ hour after preparation.

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution with a concentration 100 milligrams per milliliter.

(5) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(6) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(7) *Identity.* Proceed as directed in paragraph (b)(1)(i) of this section.

§ 455.50 Calcium novobiocin.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Calcium novobiocin is the calcium salt of a kind of novobiocin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 840 micrograms per milligram, expressed in terms of novobiocin on an anhydrous basis.

(ii) It passes the safety test.

(iii) Its loss on drying is not more than 10 percent.

(iv) Its pH in a saturated aqueous suspension containing 25 milligrams per milliliter is not less than 6.5 and not more than 8.5.

(v) Its specific rotation in an acid-methyl alcohol solution at 25° C. is not less than −50° and not more than −58°.

(vi) It demonstrates a positive color identity test.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, loss on drying, pH, specific rotation, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in 5 milliliters of absolute ethyl alcohol and then dilute with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of 1,000 micrograms (estimated) per milliliter. Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 0.5 microgram of novobiocin per milliliter (estimated).

(2) *Safety.* Proceed as directed in § 436.33 of this chapter.

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a saturated aqueous suspension prepared by suspending 25 milligrams of calcium novobiocin per milliliter.

(5) *Specific rotation.* Proceed as directed in § 455.51(b)(8).

(6) *Identity.* Proceed as directed in § 455.51(b)(9).

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

§ 455.51 Sodium novobiocin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium novobiocin is the monosodium salt of a kind of novobiocin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 850 micrograms of novobiocin per milligram, calculated on an anhydrous basis.

(ii) It passes the safety test.

(iii) Its loss on drying is not more than 6.0 percent.

(iv) Its pH in a solution containing 25 milligrams per milliliter is not less than 6.5 and not more than 8.5.

(v) Its residue on ignition is not less than 10.5 percent and not more than 12.0 percent, calculated on an anhydrous basis.

(vi) Its specific rotation in an acid-methyl alcohol solution at 25° C. is not less than −50° and not more than −58°.

(vii) It demonstrates a positive color identity test.

(viii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, loss on drying, pH, residue on ignition, specific rotation, identity and crystallinity.

(ii) Samples required on the batch: 10 packages, each containing approximately 600 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 0.5 microgram of novobiocin per milliliter (estimated).

(2) *Safety.* Proceed as directed in § 436.33 of this chapter.

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution containing 25 milligrams of sodium novobiocin per milliliter.

(5) *Residue on ignition.* Proceed as directed in § 436.207(b) of this chapter, calculating on the basis of an anhydrous sample weight.

(6) *Specific rotation.* Accurately weigh approximately 1.25 grams of the sample in a 25-milliliter glass-stoppered volumetric flask. Prepare an acid-methyl alcohol solution by diluting 1.0 milliliter of concentrated hydrochloric acid to a volume of 100 milliliters with absolute methyl alcohol and mix well. Dissolve the sample in about 15-milliliters of the acid-methyl alcohol solution. Adjust to volume with the acid-methyl alcohol solution and mix well. Proceed as directed in § 436.210 of this chapter, using a 2.0-decimeter polarimeter tube. Calculate the specific rotation on the anhydrous basis.

(7) *Identity.* (i) Using 0.1*M* aqueous sodium borate as a diluent, prepare 10 milliliters of a solution containing the equivalent of 1 milligram (approximate) of novobiocin per milliliter.

(ii) Prepare a saturated aqueous solution of *N*,2,6-trichloroquinoneimine by shaking continuously for 30 minutes in a dark bottle 25 milligrams of *N*,2,6-trichloroquinoneimine in 100 milliliters of distilled water. Let stand 2 hours after shaking. Store in the dark bottle.

(iii) Add 2.0 milliliters of the saturated *N*,2,6-trichloroquinoneimine solution to 4 milliliters of the novobiocin solution. Mix well and heat in a water bath at 37° C. for 10 minutes. The development of a blue color is a positive test for the presence of novobiocin. To 2 milliliters of the blue solution, add 2 milliliters of *N*-butyl alcohol and shake well. A green color should develop in the butyl alcohol layer. To the other 2-milliliter portion of the blue solution, add 2 milliliters of benzene (c.p.), and shake well. A pink color should be developed in the benzene layer.

(8) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

§ 455.51a Sterile sodium novobiocin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium novobiocin is the crystalline monosodium salt at a kind of novobiocin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 850 micrograms of novobiocin per milligram, calculated on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It passes the safety test.

(v) Its loss on drying is not more than 6.0 percent.

(vi) Its pH in a solution containing 25 milligrams per milliliter is not less than 6.5 and not more than 8.5.

(vii) Its residue on ignition is not less than 10.5 percent and not more than 12.0 percent calculated on an anhydrous basis.

(viii) Its specific rotation in an acid-methyl alcohol solution at 25° C. is not less than −50° and not more than −58°.

(ix) It demonstrates a positive color identity test.

(x) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, safety, loss on drying, pH, residue on ignition, specific rotation, identity, and crystallinity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 600 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 0.5 microgram of novobiocin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 10 milligrams of novobiocin per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this chapter.

(5) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution containing 25 milligrams of sodium novobiocin per milliliter.

(7) *Residue on ignition.* Proceed as directed in § 436.207(b) of this chapter, calculating on the basis of an anhydrous sample weight.

(8) *Specific rotation.* Accurately weigh approximately 1.25 grams of the sample in a 25-milliliter glass-stoppered volumetric flask. Prepare an acid-methyl alcohol solution by diluting 1.0 milliliter

of concentrated hydrochloric acid to a volume of 100 milliliters with absolute methyl alcohol and mix well. Dissolve the sample in about 15-milliliters of the acid-methyl alcohol solution. Adjust to volume with the acid-methyl alcohol solution and mix well. Proceed as directed in § 436.210 of this chapter, using a 2.0-decimeter polarimeter tube. Calculate the specific rotation on an anhydrous basis.

(9) *Identity.* (i) Using 0.1*M* aqueous sodium borate as a diluent, prepare 10 milliliters of a solution containing the equivalent of 1 milligram (approximate) of novobiocin per milliliter.

(ii) Prepare a saturated aqueous solution of *N*,2,6-trichloroquinoneimine by shaking continuously for 30 minutes in a dark bottle 25 milligrams of *N*,2,6-trichloroquinoneimine in 100 milliliters of distilled water. Let stand 2 hours after shaking. Store in the dark bottle.

(iii) Add 2.0 milliliters of the saturated *N*,2,6-trichloroquinoneimine solution to 4 milliliters of the novobiocin solution. Mix well and heat in a water bath at 37° C. for 10 minutes. The development of a blue color is a positive test for the presence of novobiocin. To 2 milliliters of the blue solution, add 2 milliliters of *N*-butyl alcohol and shake well. A green color should develop in the butyl alcohol layer. To the other 2-milliliter portion of the blue solution, add 2 milliliters of benzene (c.p.), and shake well. A pink color should develop in the benzene layer.

(10) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

§ 455.70 Rifampin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Rifampin is a red-brown powder. It is 3-(4-methylpiperazinylimino-methyl) rifamycin SV. It is very slightly soluble in water, soluble in ethyl acetate and in methyl alcohol, and freely soluble in chloroform. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram.

(ii) It passes the safety test.

(iii) Its loss on drying is not more than 2 percent.

(iv) Its pH is not less than 4.0 and not more than 6.0 in a 1 percent aqueous suspension.

(v) When calculated on the anhydrous basis, its absorptivity at 475 nanometers is 100±4 percent of that of the rifampin working standard, similarly treated.

(vi) It passes the identity test.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, loss on drying, pH, absorptivity, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient methyl alcohol to give a stock solution containing 1.0 milligram of rifampin per milliliter (estimated). Further dilute an aliquot of the stock solution with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 5.0 micrograms of rifampin per milliliter (estimated).

(2) *Safety.* Proceed as directed in § 436.33 of this chapter.

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter, except dry the sample for 4 hours.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a 1 percent aqueous suspension.

(5) *Absorptivity.* Determine the absorbance of the sample and standard solutions in the following manner: Dissolve approximately 100 milligrams each of the sample and standard in a 100-milliliter volumetric flask containing 50 milliliters of absolute methyl alcohol, and dilute to volume with absolute methyl alcohol. Transfer a 2-milliliter aliquot to a 100-milliliter volumetric flask, and dilute to volume with 1 percent potassium phosphate buffer, pH 6.0, as listed in § 436.101(a)(1) of this chapter. Using a suitable spectrophotometer equipped with a 1-centimeter cell, immediately determine the absorption of each solution at 475 nanometers with the blank containing the same proportion of solution 1 and methyl alcohol as the sample and standard solutions. Calculate the absorptivity as follows:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{milligrams standard} \times (100 - m_1)}{\text{Absorbance of standard} \times \text{milligrams sample} \times (100 - m_2)} \times 100$$

where

m_1 = percent moisture in standard;
m_2 = percent moisture in sample.

(v) It passes the safety test.

(vi) It contains no histamine nor histamine-like substances.

(vii) Its moisture content is not less than 16 percent nor more than 20 percent.

(viii) Its pH is an aqueous solution containing 10 milligrams per milliliter is not less than 3.8 nor more than 5.6. If it is packaged for dispensing, when reconstituted as directed in the labeling, its pH is not less than 4.0 nor more than 7.0.

(ix) It passes the identity test.

(x) Its residue on ignition is less than 1 percent.

(xi) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for spectinomycin content, microbiological activity, sterility, pyrogens, safety, histamine, moisture, pH, identity, residue on ignition, and crystallinity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: eight packages, each containing approximately 300 milligrams and two containing not less than 3 grams.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Spectinomycin content (vapor phase chromatography).* Proceed as directed in § 436.307 of this chapter; and also, if the batch is packaged for dispensing prepare the sample for assay as follows: Reconstitute the vial as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container, or if the labeling specifies the amount of spectinomycin content in a given volume of the resultant preparation remove an accurately measured representative portion from the container. Dilute the

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b)(3) of that section, except use a 4 percent solution of the sample in chloroform and 0.1-millimeter matched absorption cells.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

§ 455.80a Sterile spectinomycin hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile spectinomycin hydrochloride is the pentahydrated dihydrochloride salt of decahydro-4a, 7, 9-trihydroxy - 2 - methyl - 6,8-bis(methylamino) 4H - pyrano[2,3 - b][1,4]benzodioxin-4-one. It is so purified and dried that:

(i) Its spectinomycin content is not less than 603 micrograms per milligram. If it is packaged for dispensing, its spectinomycin content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of spectinomycin that it is represented to contain.

(ii) Its microbiological activity is not less than 603 micrograms of spectinomycin per milligram.

(iii) It is sterile.

(iv) It is nonpyrogenic.

sample with water to a concentration equivalent to about 20 milligrams per milliliter of spectinomycin. Transfer 1.0 milliliter of the diluted sample to a 25-milliliter glass-stoppered Erlenmeyer flask and dry by lyophilization. Proceed as directed in § 436.307(d)(1)(ii) of this chapter. Calculate the spectinomycin content as follows:

$$\text{Milligrams of spectinomycin per dose} = \frac{R_u \times W_s \times D \times f}{R_s}$$

where:

R_u = Area of spectinomycin sample peak (at a retention time equal to that observed for the spectinomycin standard) / Area of internal standard peak

R_s = Area of the spectinomycin standard peak / Area of internal standard peak

W_s = Weight of the spectinomycin working standard in milligrams;

D = Dilution of the spectinomycin dose;

f = Potency of the spectinomycin working standard in milligrams of spectinomycin per milligram.

(2) *Microbiological activity (microbiological turbidimetric assay).* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 30.0 micrograms of spectinomycin per milliliter (estimated).

(3) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (a)(1) of that section.

(4) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 50 milligrams of spectinomycin base per milliliter.

(5) *Safety.* Proceed as directed in § 436.33 of this chapter.

(6) *Histamine.* Proceed as directed in § 436.35 of this chapter.

(7) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(8) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter, except, if it is packaged for dispensing, use the suspension obtained after reconstituting the drug as directed in the labeling.

(9) *Identity test.* Proceed as directed in § 436.211 of this chapter, using the method described in paragraph (b)(2) of that section.

(10) *Residue on ignition.* Proceed as directed in § 436.207 of this chapter, using the method described in paragraph (b) of that section.

(11) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

§ 455.85 Vancomycin hydrochloride.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Vancomycin hydrochloride is the hydrochloride salt of a kind of vancomycin or a mixture of two or more such salts. It is soluble in water and moderately soluble in dilute methyl alcohol. It is insoluble in higher alcohols, acetone, and ether. It is so purified and dried that:

(i) It contains not less than 900 micrograms of vancomycin per milligram, calculated on an anhydrous basis.

(ii) It passes the safety test.

(iii) Its moisture content is not more than 5 percent.

(iv) Its pH in an aqueous solution containing 50 milligrams per milliliter is not less than 2.5 and not more than 4.5.

(v) It contains not more than 15 percent of factor A.

(vi) It gives a positive identity test for vancomycin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, moisture, pH, factor A content, and identity.

(ii) Samples required: 12 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample of approximately 30 milligrams in sufficient sterile distilled water to give a stock solution of 1 milligram per milliliter (estimated). Further dilute an aliquot of the stock solution with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 10 micrograms of vancomycin per milliliter (estimated).

(2) *Safety.* Proceed as directed in § 436.33 of this chapter.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution containing 50 milligrams per milliliter.

(5) *Identity and factor A content.* Proceed as directed in § 455.85a(b)(7).

§ 455.85a Sterile vancomycin hydrochloride.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Sterile vancomycin hydrochloride is the hydrochloride salt of a kind of vancomycin or a mixture of two or more such salts. It is soluble in water and moderately soluble in dilute methyl alcohol. It is insoluble in higher alcohols, acetone, and ether. It is so purified and dried that:

(i) It contains not less than 900 micrograms of vancomycin per milligram, calculated on an anhydrous basis. If it is packaged for dispensing, its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of vancomycin that it is represented to contain.

(ii) It is sterile.

(iii) It passes the safety test.

(iv) It is nonpyrogenic.

(v) Its moisture content is not more than 5 percent.

(vi) Its pH in an aqueous solution containing 50 milligrams per milliliter is not less than 2.5 and not more than 4.5.

(vii) Its heavy metals content is not more than 30 parts per million.

(viii) It contains not more than 15 percent of factor A.

(ix) It gives a positive identity test for vancomycin.

(2) *Packaging.* In addition to the requirements of § 432.1 of this chapter, if it is packaged for dispensing, the vancomycin content of each immediate container is 500 milligrams of vancomycin.

(3) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(4) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, safety, pyrogens, moisture, pH, heavy metals, factor A content, and identity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use as an ingredient in the manufacture of another drug:

(*1*) For all tests except sterility: 12 packages, each containing approximately 500 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 12 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample of approximately 30 milligrams in sufficient sterile distilled water to give a stock solution of 1 milligram per milliliter; and also if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to give a stock solution of 1 milligram per milliliter. Further dilute an aliquot of the stock solution with solution 4 to the reference concentration of 10.0 micrograms of vancomycin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use sterile distilled water in lieu of diluting fluid A.

(3) *Safety.* Proceed as directed in § 436.33 of this chapter.

(4) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 5 milligrams of vancomycin per milliliter.

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution containing 50 milligrams per milliliter.

(7) *Identity and factor A content—(i) Preparation of the chromatogram—(a) Equipment.* (*1*) Chromatographic paper (Whatman No. 1 untreated filter paper).

(*2*) Equipment for descending paper chromatography (Mitchell tank).

(*b*) *Preparations of solutions—*(*1*) *Factor A.* Prepare a solution in distilled water to contain 1.33 milligrams of factor A per milliliter and further dilute with distilled water to prepare solutions containing 0.1 and 0.2 milligram of factor A per milliliter.

(*2*) *Vancomycin working standard solution.* Prepare a solution in distilled water to contain 1.33 milligrams of vancomycin per milliliter.

(*3*) *Known mixture of factor A and vancomycin.* Prepare a solution in distilled water to contain 0.2 milligram of factor A and 1.13 milligrams of vancomycin (estimated) per milliliter.

(*4*) *Sample.* Prepare two solutions of the sample in distilled water, each to contain 1.33 milligrams of vancomycin (estimated) per milliliter.

(*5*) *Solvent mixture.* Mix 300 milliliters of butyl alcohol, 150 milliliters of pyridine, and 200 milliliters of water in a large separatory funnel and shake well for 3 minutes. Let stand at room temperature. There should be no separation of layers.

(*c*) *Procedure.* Saturate the atmosphere in the tank with vapors of the solvent mixture by placing 10 milliliters of the mixture in a trough in the bottom of the tank and closing tightly for 15 minutes. Prepare a sheet of chromatographic paper (8 inches x 8 inches) by carefully drawing a line of origin with a pencil 2 inches from one of the edges. Fold the paper along a straight line 1½ inches from the same edge of the paper. Starting 1 inch from the left-hand edge, establish points at 1-inch intervals along the line of origin on which to apply the solutions. Using a micropipette, apply the factor A solutions, the vancomycin solution, the known mixture solution, and the sample solutions by placing 5 microliters of each on separate spots. Properly identify the locations of the spots but avoid unnecessary handling of the paper. Allow the spots to dry spontaneously. Suspend the paper in the chamber so that the edge nearest the fold can be conveniently immersed in the solvent mixture contained in the top trough. Immerse the paper across its entire width to a depth sufficient to assure contact with the solvent mixture during the entire development time. Close the chamber tightly and allow the chromatograph to develop at room temperature for 6½ to 7 hours. Remove the paper and allow it to dry completely.

(ii) *Development by bioautograph—*(*a*) *Preparation of test organism (spore suspension).* The test organism is *Bacillus subtilis* (ATCC 6633),[1] test organism H, prepared as described in § 436.103 of this chapter, using the method described in paragraph (b)(2) of that section.

(*b*) *Preparation of plates—*(*1*) *Base layer.* Add 42 milliliters of medium 2 described in § 436.102(b)(2) of this chapter to each Petri dish (25 millimeters x 150 millimeters) and allow to harden on a flat, level surface. To prevent condensation of excess moisture, raise the tops slightly while the agar hardens.

(*2*) *Seed layer.* Melt nutrient agar medium 2 described in § 436.102(b)(2) of this chapter. Accurately measure a sufficient quantity of the melted agar, cool to 48° C., and add the appropriate quantity of the spore suspension prepared as described in paragragh (b)(7)(ii)(*a*) of this section. Swirl the flask of inoculated agar to obtain a homogeneous suspension. Add 8 milliliters of this inoculated agar to each plate, spread evenly, and allow to harden on a flat, level surface. For accurate results, it is necessary to obtain uniform distribution of the agar over the entire surface of the plates.

(*c*) *Assay.* For each spot on the paper described in paragraph (b)(7)(i)(*c*) of this section, cut a strip 1.5 centimeters by approximately 14 centimeters with the center of each strip centered about the line of descent of the spot. Place all strips on plates with the aid of forceps within as short a period of time as possible. Use maximum spacing between strips. Insure complete contact so that the entire strip becomes uniformly moistened. Allow to stand for 30 minutes. Remove the strips and identify each strip location on the Petri dish. Incubate the plates for 16–18 hours at 37° C. Any zone of inhibition corresponding to factor A in the sample must not be greater than that of the 0.2 milligram-per-milliliter factor A standard. Also, the two areas of inhibition for the sample due to the presence of factor A and vancomycin must compare to the corresponding two areas of inhibition of the known mixture in their respective distances from their origins.

(*8*) *Heavy meals.* Proceed as directed in § 436.208 of this chapter.

Subpart B—Oral Dosage Forms

§ 455.110 Chloramphenicol capsules.

(a) *Requirements for certification—*(*1*) *Standards of identity, strength, quality, and purity.* Chloramphenicol capsules are composed of chloramphenicol with or without one or more suitable and harmless diluents and lubricants. Each capsule contains 50, 100, or 250 milligrams of chloramphenicol. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of chloramphenicol that it is represented to contain. The chloramphenicol used conforms to the standards prescribed by § 455.10(a)(1).

(*2*) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(*3*) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The chloramphenicol used in making the batch for potency, safety, pH, specific rotation, melting range, absorptivity, and crystallinity.

(*b*) The batch for potency.

(ii) Samples required:

(*a*) The chloramphenicol used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay; potency.* Use either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(*1*) *Microbiological turbidimetric assay.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed, glass blender jar containing 100 milliliters of 95 percent ethyl alcohol. Blend for 2 minutes. Then add 400 milliliters of 1 percent potassium phosphate buffer, pH 6.0 (solution 1), and blend again for 2 minutes. Remove an aliquot and further dilute with solution 1 to the reference concentration of 2.5 micrograms of chloramphenicol per milliliter (estimated).

(*2*) *Spectrophotometric assay—*(i) *Preparation of working standard solution.* Dissolve approximately 50 milligrams of the working standard in 100 milliliters of distilled water. Warm if necessary to hasten dissolution. Transfer 10 milliliters into a 250-milliliter volumetric flask and fill to volume with distilled water.

(ii) *Procedure.* Place the contents of 10 capsules into a 250-milliliter volumetric flask. Add 50 milliliters of pure methyl alcohol to the flask and shake for at least 1 minute. Fill to volume with distilled water and mix thoroughly. Withdraw an aliquot and dilute with sufficient distilled water to give a concentration of 20 micrograms per milliliter. Using a suitable spectrophotometer equipped with a 1.0-centimeter cell and distilled water as the blank, determine the absorbance of the working standard and sample solutions at 278 nanometers. Calculate the potency as follows:

$$\text{Milligrams per capsule} = \frac{\text{Absorbance of sample} \times \text{labeled potency per capsule in milligrams}}{\text{Absorbance of standard}}$$

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

§ 455.111 Chloramphenicol palmitate oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chloramphenicol palmitate oral suspension is chloramphenicol palmitate and one or more suitable and harmless buffer substances, suspending agents, preservatives, colorings, and flavorings suspended in a suitable and harmless vehicle. Each milliliter contains 31.25 milligrams of chloramphenicol. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of chloramphenicol that it is represented to contain. Its pH is not less than 4.5 nor more than 7.0. Its content of polymorph A crystals does not exceed 10 percent. The chloramphenicol palmitate used conforms to the standards prescribed by § 455.11(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The chloramphenicol palmitate used in making the batch for potency, safety, melting range, specific rotation, and crystallinity.

(*b*) The batch for potency, pH, and content of polymorph A crystals.

(ii) Samples required:

(*a*) The chloramphenicol palmitate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Preparation of chloramphenicol working standard solution.* Dissolve approximately 50 milligrams of the chloramphenicol working standard, accurately weighed, in a 100-milliliter volumetric flask with absolute ethyl alcohol. Fill to volume with absolute ethyl alcohol. Transfer 10.0 milliliters into a 250-milliliter volumetric flask and fill to volume with absolute ethyl alcohol.

(ii) *Procedure.* Transfer an accurately measured amount of the suspension, equivalent to about 125 milligrams of chloramphenicol, to a separatory funnel. Add 10 milliliters of water and then extract completely with 25-milliliter portions of chloroform, allowing the layers to separate and filtering each successive chloroform extract through a pledget of cotton into a 250-milliliter volumetric flask, then add chloroform to volume, and mix. Pipette 4 milliliters of the clear chloroform solution into a 100-milliliter volumetric flask, dilute with ethyl alcohol to volume, and mix. Using a suitable spectrophotometer equipped with a 1.0-centimeter cell and a blank composed of 1.0 milliliter of chloroform, U.S.P. diluted to 25 milliliters with absolute ethyl alcohol, determine the absorbance of the chloroform working standard solution at 276 nanometers and that of the sample solution at 271 nanometers. Calculate the potency of the sample as follows:

$$\text{Milligrams of chloramphenicol per milliliter} = \frac{\text{Absorbance of sample} \times \text{labeled potency per milliliter in milligrams}}{\text{Absorbance of standard}}$$

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted sample.

(3) *Content of polymorph A crystal.*—(i) *Preparation of standards*—(*a*) *Standard containing 20 percent of polymorph A.* Prepare a thoroughly mixed, dry powder composed by weight of 1 part of polymorph A crystals of chloramphenicol palmitate and 4 parts of nonpolymorph A crystals of chloramphenicol palmitate.

(*b*) *Standard containing 10 percent of polymorph A.* Prepare a thoroughly mixed, dry powder composed by weight of 1 part of polymorph A crystals of chloramphenicol palmitate and 9 parts of nonpolymorph A crystals of chloramphenicol palmitate.

(ii) *Preparation of sample.* Place 20 milliliters of thoroughly mixed oral suspension into a 50-milliliter centrifuge tube. Add 20 milliliters of water and mix. Centrifuge for 10 to 15 minutes at a speed not less than 18,000 revolutions per minute. Decant the supernatant liquid. Wash the residue as follows: Add 2 milliliters of water to the residue, mix to make paste, add 18 milliliters of water, and mix thoroughly. Centrifuge, decant the supernatant liquid, and wash the residue two more times. Remove the washed residue from the centrifuge tube and dry it at least 14 hours in a vacuum desiccator at room temperature.

(iii) *Procedure.* Weigh 150 to 200 milligrams of liquid petrolatum into an agate mortar and add about 100 milligrams of standard or sample. Mix with a small spatula and then mull thoroughly with a pestle until a uniform consistency is obtained. Adjust a suitable infrared spectrophotometer so that 100 percent transmittance is recorded over the range of 11.0 to 13.0 microns. Use two rock salt plates as an absorption cell. Place a small drop of the mull in the center of one of the plates. Gently put the other plate on the mull and slowly squeeze the plates together to spread the mull uniformly. Clamp the two plates firmly together in a metal cell holder. Examine the assembled cell by holding it up to the light. It should appear smooth and free of any air bubbles and when placed in the instrument it should give a percent transmittance of 20 to 30 percent at 12.3 microns. Place the cell in the infrared spectrophotometer and record the absorption spectrum from 11.0 to 13.0 microns.

(iv) *Treatment of spectra*—(*a*) *Standard containing 20 percent of polymorph A.* Determine by inspection of the recorded spectrum the exact wavelengths of minimum absorption at approximately 11.3 and 12.65 microns. Also determine by inspection the exact wavelengths of maximum absorption at approximately 11.65 and 11.86 microns. In the following subdivision, references to these four nominal wavelengths are to the exact wavelengths observed on the particular instrument being used.

(*b*) *Standard containing 10 percent of polymorph A.* Draw a straight baseline between the minima occurring at 11.3 and 12.65 microns. Draw straight lines at 11.65 and 11.86 microns intersecting both the recorded spectrum and the baseline. Obtain the corrected absorbances at 11.65 and 11.86 microns and calculate the absorbance ratios as follows:

$$\text{Absorbance ratio} = \frac{{}^{s}11.65 - {}^{B}11.65}{{}^{s}11.86 - {}^{B}11.86}$$

where:

$^{s}11.65$ = Absorbance value of recorded spectrum at 11.65 microns;

$^{B}11.65$ = Absorbance value at point of intersection of the 11.65-micron line with the baseline;

$^{s}11.86$ = Absorbance value of recorded spectrum at 11.86 microns;

$^{B}11.86$ = Absorbance value at point of intersection of the 11.86-micron line with the baseline.

(*c*) *Sample.* Proceed as described in paragraph (b)(3)(iv)(*b*) of this section.

(v) *Calculation.* The absorbance ratio of the sample must be greater than the absorbance ratio of the standard containing 10 percent of polymorph A.

§ 455.120 Cycloserine capsules.

(a) *Requirements for certification*—(1) *Standards of identity, quality, and purity.* Cycloserine capsules are capsules composed of crystalline cycloserine, with or without one or more suitable and harmless buffer substances, diluents, binders, and lubricants. Each capsule contains 250 milligrams of cycloserine. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cycloserine that it is represented to contain. The loss on drying is not more than 1.0 percent. The cycloserine used conforms to the standards prescribed by § 455.20(a)(1).

(2) *Labeling.* In addition to the labeling prescribed by § 432.5 of this chapter, the labeling of each package shall bear a warning to the effect that the drug is to be used in patients with tuberculosis who fail to respond to treatment with isoniazid, streptomycin, paraaminosalicylic acid, viomycin, pyrazinamide, or combinations of these drugs, and that the drug may cause serious reactions such as convulsive seizures and mental disturbances.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) Cycloserine used in making the batch for potency, safety, loss on drying, pH, residue on ignition, crystallinity, and identity.

(*b*) The batch for cycloserine content and loss on drying.

(ii) Samples required:

(*a*) Cycloserine used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: Minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Using the cycloserine working standard as the standard of comparison, assay for potency by either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(i) *Chemical colorimetric assay*—(*a*) *Reagents.* (*1*) Acetic acid—1.0*N* solution.

(*2*) Sodium hydroxide—4.0*N* and 0.1*N* solutions.

(*3*) Sodium nitroprusside—4.0 percent solution: Dissolve 4.0 grams in sufficient distilled water to make 100.0 milliliters. Mix well. Store in amber bottle.

(*4*) Oxidized nitroprusside reagent—Mix equal parts of the 4.0 percent sodium nitroprusside solution and 4.0*N* sodium hydroxide, and let stand for 1 hour before using. Prepare daily and store in amber bottle.

(*5*) Cycloserine standard solution—dilute an appropriate-sized aliquot of the stock standard solution, prepared as directed in § 455.20(b)(1)(i)(*a*), in 0.1*N* sodium hydroxide to obtain a working standard solution containing 100 micrograms of cycloserine per milliliter.

(*b*) *Procedure.* Transfer the contents of 10 capsules into a 1,000-milliliter volumetric flask. Add 0.1*N* sodium hydroxide to dissolve the sample, and add sufficient 0.1*N* sodium hydroxide to measure 1,000 milliliters. Mix well and filter. Dilute an aliquot of the filtrate with sufficient 0.1*N* sodium hydroxide to give a concentration of 0.1 milligram per milliliter (estimated) and mix well. Pipette exactly 1.0 milliliter of the working standard solution and 1.0 milliliter of the sample solution into separate test tubes. Add exactly 3.0 milliliters of 1.0*N* acetic acid and exactly 1.0 milliliter of oxidized nitroprusside reagent to each of the test tubes; then mix thoroughly. Allow the tubes to stand at room temperature for 10 to 15 minutes, in order that maximum color intensity may develop. Using a reagent blank, determine the absorbance of the solutions at 625 nanometers in a suitable spectrophotometer.

Calculation:

$$\text{Milligrams of cycloserine per capsule} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times \text{Labeled potency per capsule in milligrams.}$$

(ii) *Microbiological turbidimetric assay.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules in a high-speed glass blender with sufficient sterile distilled water to give a stock solution of convenient concentration. Blend 3 to 5 minutes. Further dilute the stock solution with sterile distilled water to the reference concentration of 50 micrograms of cycloserine per milliliter (estimated).

(*2*) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

§ 455.150 Calcium novobiocin oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Calcium novobiocin oral suspension is a suspension containing calcium novobiocin and one or more suitable and harmless diluents, preservatives, suspending agents, surfactants, flavorings, and colorings in purified water. Each milliliter contains 25 milligrams of novobiocin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of novobiocin that it is represented to contain. The pH is not less than 6.0 and not more than 7.5. The calcium novobiocin used conforms to the standards prescribed by § 455.50(a)(1)(i), (ii), (iv), (v), (vi), and (vii). If sodium novobiocin is reacted with a suitable calcium salt to form calcium novobiocin, the sodium novobiocin used conforms to the standards prescribed by § 455.51(a)(1)(i), (ii), (iv), (v), (vi), (vii), and (viii).

(2) *Labeling.* It shall be labeled in accordance with § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The calcium novobiocin used in making the batch for potency, safety, pH, crystallinity, identity, and specific rotation. If sodium novobiocin is used in making the batch: Potency, safety, pH, residue on ignition, specific rotation, identity, and crystallinity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The calcium novobiocin or the sodium novobiocin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: Minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Remove a representative sample of the sirup with a suitable syringe and place into a high-speed glass blender with sufficient absolute ethyl alcohol to give a concentration (estimated) of 1,000 micrograms per milliliter. Blend for 3 to 5 minutes. Further dilute with 10 percent potassium phosphate buffer, pH 1.0 (solution 6), to the reference concentration of 0.5 microgram of novobiocin per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted suspension.

§ 455.151 Sodium novobiocin oral dosage forms.

§ 455.151a Sodium novobiocin tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium novobiocin tablets are tablets that contain sodium novobiocin, with or without one or more suitable and harmless buffer substances, diluents, binders, and lubricants. Each tablet contains 125 milligrams or 250 milligrams of novobiocin. The 125-milligram tablet contains 375 milligrams of sulfamethizole. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of novobiocin that it is represented to contain. Its loss on drying is not more than 3 percent. The tablets disintegrate within 1 hour. The sodium novobiocin used conforms to the standards prescribed by § 455.51(a)(1).

(2) *Labeling.* It shall be labeled in accordance with § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) Sodium novobiocin used in making the batch for potency, safety, loss on drying, pH, residue on ignition, specific rotation, identity, and crystallinity.

(*b*) The batch for potency, loss on drying, disintegration time.

(ii) Samples required:

(*a*) Sodium novobiocin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Blend a representative number of tablets in a high-speed glass blender with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute the stock solution with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 0.5 microgram of novobiocin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter, using the method described in paragraph (e)(1) of that section.

§ 455.151b Sodium novobiocin capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium novobiocin capsules are gelatin capsules containing sodium novobiocin with a suitable and harmless filler and with or without a binder and a lubricant. Each capsule contains 100 milligrams or 250 milligrams of novobiocin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of novobiocin that it is represented to contain. The loss on drying is not more than 6.0 percent. The sodium novobiocin used conforms to the standards prescribed by § 455.51(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The sodium novobiocin used in making the batch for potency, safety, loss on drying, pH, residue on ignition, specific rotation, crystallinity, and identity.

(b) The batch for potency and loss on drying.

(ii) Samples required:

(a) The sodium novobiocin used in making the capsules: 10 packages, each containing approximately 500 milligrams.

(b) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules in a high-speed glass blender with 1.0 milliliter of polysorbate 80 and sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Blend 3 to 5 minutes. Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 0.5 microgram of novobiocin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

§ 455.170 Rifampin capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Rifampin capsules are gelatin capsules containing rifampin with a suitable and harmless filler and with or without binders, lubricants, and stabilizers. Each capsule contains 300 milligrams of rifampin. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of rifampin that it is represented to contain. Its loss on drying is not more than 3.0 percent. The rifampin used conforms to the standards prescribed by § 455.70(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The rifampin used in making the batch for potency, safety, loss on drying, pH, absorptivity, identity, and crystallinity.

(b) The batch for potency and loss on drying.

(ii) Samples required:

(a) The rifampin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) Potency. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar containing 200 milliliters of methyl alcohol and blend for 3 minutes. Add 300 milliliters of 1 percent potassium phosphate buffer, pH 6.0 (solution 1), and blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 1 to the reference concentration of 5.0 micrograms of rifampin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

§ 455.185 Vancomycin hydrochloride for oral solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Vancomycin hydrochloride for oral solution is vancomycin hydrochloride packaged in a suitable dispensing container. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of grams of vancomycin that it is represented to contain. Its moisture content is not more than 5 percent. When reconstituted as directed in the labeling, its pH is not less than 2.5 and not more than 4.5. The vancomycin hydrochloride used conforms to the standards prescribed by § 455.85.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assay on:

(a) The vancomycin hydrochloride used in making the batch for potency, safety, moisture, pH, factor A content, and identity.

(b) The batch for potency, moisture, and pH.

(ii) Samples required:

(a) The vancomycin hydrochloride used in making the batch: 12 packages, each containing approximately 500 milligrams.

(b) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Empty the contents into an accurately measured volume of distilled water as directed in the labeling of the drug. Further dilute an aliquot with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 10 micrograms of vancomycin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

Subpart C—Injectable Dosage Forms

§ 455.210 Chloramphenicol injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chloramphenicol injection is chloramphenicol, with or without one more suitable and harmless buffer substances, dissolved in one or more suitable and harmless solvents. Each milliliter contains 250 milligrams of chloramphenicol. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of chloramphenicol that it is represented to contain. It is sterile. It is nonpyrogenic. It passes the safety test. It contains no histamine nor histamine-like substances. Its pH is not less than 4.7 and not more than 5.0. The chloramphenicol used conforms to the standards prescribed by § 455.10a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The chloramphenicol used in making the batch for potency, pH, specific rotation, melting range, absorptivity, and crystallinity.

(b) The batch for potency, sterility, pyrogens, safety, histamine, and pH.

(ii) Samples required:

(a) The chloramphenicol used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of eight immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative aliquot of the sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a convenient concentration. Further dilute with solution 1 to the reference concentration of 2.5 micrograms of chloramphenicol per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except add the contents of each container directly to the dry filter, thus eliminating the preliminary solubilization step.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 5 milligrams per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this chapter.

(5) *Histamine.* Proceed as directed in § 436.35 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted drug.

§ 455.251 Sodium novobiocin for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium novobiocin for injection is sodium novobiocin with or without one or more suitable solubilizing agents, preservatives, and diluents. Each vial contains 500 milligrams of novobiocin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of novobiocin that it is represented to contain. It is sterile, nonpyrogenic, and nontoxic. Its loss on drying is not more than 6.0 percent. Its pH, when reconstituted as directed in the labeling, is not less than 6.5 and not more than 8.5. The sodium novobiocin used conforms to the

standards prescribed by § 455.51a(a)(1)(i), (iii), (iv), (v), (vi), (vii), and (viii).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The sodium novobiocin used in making the batch for potency, loss on drying, pH, residue on ignition, specific rotation, crystallinity, and identity.

(*b*) The batch for potency, sterility, pyrogens, safety, loss on drying, and pH.

(ii) Samples required:

(*a*) The sodium novobiocin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 0.5 microgram of novobiocin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32 of this chapter, using a solution containing 10 milligrams of novobiocin per milliliter.

(4) *Safety.* Proceed as directed in § 436.33 of this chapter.

(5) *Loss on drying.* Proceed as directed in §436.200 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using the sample after reconstituting as directed in the labeling.

§ 455.280a Sterile spectinomycin hydrochloride.

The requirements for certification and the tests and methods of assay for sterile spectinomycin hydrochloride packaged for dispensing are described in § 455.80a.

§ 455.285a Sterile vancomycin hydrochloride.

The requirements for certification and the tests and methods of assay for sterile vancomycin hydrochloride packaged for dispensing are described in § 455.85a.

Subpart D—Ophthalmic Dosage Forms

§ 455.310 Chloramphenicol ophthalmic dosage forms.

§ 455.310a Chloramphenicol ophthalmic solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chloramphenicol ophthalmic solution contains in each milliliter 5 milligrams of chloramphenicol with or without one or more suitable and harmless preservatives, buffer substances, and surfactants in an aqueous solution. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of chloramphenicol that it is represented to contain. It is sterile. Its pH is not less than 3 nor more than 6; however, if the solution is buffered, its pH is not less than 7.0 nor more than 7.5. The chloramphenicol used conforms to the standards prescribed by § 455.10(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The chloramphenicol used in making the batch for potency, safety, pH, specific rotation, melting range, absorptivity, and crystallinity.

(*b*) The batch for potency, sterility, and pH.

(ii) Samples required:

(*a*) The chloramphenicol used in making the batch: 10 containers, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of five immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(i) *Microbiological turbidimetric assay.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative aliquot of the sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 2.5 micrograms of chloramphenicol per milliliter (estimated).

(ii) *Spectrophotometric assay.* Dilute a 1-milliliter aliquot of the sample in sufficient distilled water to make a solution containing 20 milligrams of chloramphenicol per milliliter. Dissolve an accurately weighed portion of the working standard in sufficient distilled water to give a solution containing 20 milligrams per milliliter. Warm if necessary to hasten solution of the working standard. Cool. Using a suitable spectrophotometer and distilled water as the blank, determine the absorbance of the sample and standard solutions at 278 nanometers. Calculate the potency of the sample as follows:

$$\text{Milligrams of chloramphenicol per milliliter} = \frac{\text{Absorbance of sample} \times \text{labeled potency per milliliter in milligrams}}{\text{Absorbance of standard}}$$

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted solution.

§ 455.310b Chloramphenicol ophthalmic.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chloramphenicol ophthalmic is chloramphenicol, with or without one or more suitable and harmless preservatives, buffer substances, and diluents. It may contain cortisone hydrocortisone, or a suitable ester of cortisone or hydrocortisone. It is sterile. The chloramphenicol is of such quantity that when prepared as directed in its labeling the potency of such solution or suspension is not less than 1.0 milligram per milliliter and maintains its labeled potency after it has been kept for 10 days at room temperature. Such solution or suspension has a pH of 7.3±0.2. The chloramphenicol used conforms to the requirements of § 455.10b(a)(1), except paragraph (a)(1)(ii), (iv), and (v) of that section. Each other ingredient used, if its name is recognized in the U. S. P. or N. F., conforms to the standards prescribed therefore by such official compendium.

(2) *Packaging.* The immediate container of chloramphenicol ophthalmic shall be a tight container as defined by the U. S. P. Its closure shall be one through which a hypodermic needle cannot be introduced. The composition of the immediate container and closure shall be such as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded. Each such container may be packaged in combination with a container of the solvent, sterile distilled water U. S. P.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its label or labeling as hereinafter indicated, the following:

(i) On the outside wrapper or container and the immediate container:

(*a*) The statement "Expiration date _____", the blank being filled in with the date that is 60 months after the month during which the batch was certified.

(*b*) If it is packaged in combination with a container of a solvent, the statement "*Warning*—Not for injection".

(ii) If it contains one of the active ingredients specified in paragraph (a) (1) of this section, after the name "chloramphenicol ophthalmic", wherever it appears, the name of such ingredient, in juxtaposition with such name.

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch of chloramphenicol ophthalmic shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, the batch mark and (unless previously submitted) the date on which the latest assay of the chloramphenicol used in making such batch was completed, the number of milligrams of chloramphenicol in each immediate container, the date on which the latest assay of the drug comprising such batch was completed, the quantity of each other ingredient used in making the batch, and a statement that each such ingredient conforms to the requirements prescribed therefor by this section. If such batch or any part thereof is to be packaged with a solvent, such request shall also be accompanied by a statement that such solvent conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided in paragraph (a) (4) (iv) of this section, such person shall submit in connection with his request results of the tests and assays listed after each of the following, made by him on an accurately representative sample of:

(*a*) The batch: potency, sterility, and pH of the solution or suspension prepared as directed in its labeling.

(*b*) The chlorainphenicol used in making the batch; potency, toxicity, pH, specific rotation, melting point, and absorptivity.

(iii) Except as otherwise provided by paragraph (a) (4) (iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples or the following:

(*a*) The batch:

(*1*) For all tests except sterility: One immediate container for each 5,000 immediate containers in the batch, but in no case less than five immediate containers.

Such samples shall be collected by taking single immediate containers at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(*2*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(*b*) The chloramphenicol used in making the batch; 10 packages, each containing approximately equal portions of not less than 300 milligrams, packaged in accordance with the requirements of § 455.10b(a) (2).

(*c*) In case of an initial request for certification, each other ingredient used in making the batch; one package of each containing approximately 5.0 grams, except if cortisone acetate is used, such package shall contain approximately 100 milligrams.

(iv) No result referred to in paragraph (a) (4) (ii) (*b*) of this section, and no sample referred to in paragraph (a) (4) (iii) (*b*) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(i) *Microbiological turbidimetric assay.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dilute an accurately measured representative aliquot of the sample with sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a convenient concentration. Further dilute with solution 1 to the reference concentration of 2.5 micrograms of chloramphenicol per milliliter (estimated).

(ii) *Spectrophotometric assay.* Reconstitute the sample as directed in the labeling and dilute a 1.0-milliliter aliquot in sufficient distilled water to give a solution containing 20 micrograms of chloramphenicol per milliliter. Dissolve an accurately weighed portion of the working standard in sufficient distilled water to give a solution containing 20 micrograms per milliliter. Using a suitable spectrophotometer and distilled water as the blank, determine the absorbance of the sample and standard solutions at 278 nanometers. Calculate the potency of the sample as follows:

$$\text{Milligrams of chloramphenicol per milliliter} = \frac{\text{Absorbance of sample} \times \text{labeled potency per milliliter in milligrams}}{\text{Absorbance of standard}}$$

The potency of chloramphenicol ophthalmic is satisfactory if it contains not less than 90 percent and not more than 130 percent of the number of milligrams that it is represented to contain.

(2) *Sterility.* Use entire contents, and proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e) (1) of that section, except if it contains a corticoid use 0.5 milliliter of the suspension prepared according to label directions and proceed as directed in paragraph (e) (2) of that section.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

§ 455.310c Chloramphenicol ointment (chloramphenicol cream).

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chloramphenicol ointment is chloramphenicol in a suitable and harmless ointment base, with or without suitable and harmless buffer substances, dispersing and suspending agents. It may contain cortisone or a suitable derivative of cortisone. If such base is water-miscible, it shall contain a suitable and harmless preservative. Its potency is not less than 1.0 milligram per gram. If it is intended for ophthalmic use, it is sterile. The chloramphenicol used conforms to the requirements of § 455.10b(a) (1) except § 455.10b(a) (1) (ii), (iii), (iv), and (v) of that section. The chloramphenicol used in making the chloramphenicol ophthalmic ointment conforms to the requirements of § 455.10b(a) (1) except § 455.10b(a) (1) (iv) and (v). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* Unless it is packaged in a single dose container, chloramphenicol ointment shall be packaged in collapsible tubes, which shall be well-closed containers as defined by the U.S.P., and shall not be larger than the ⅛-ounce size if such ointment is represented for ophthalmic use, and in no case larger than the 2-ounce size, except that if it is labeled solely for hospital use it may be packaged in immediate containers of glass which meet the test for tight containers as defined by the U. S. P. The composition of the immediate container and closure shall be such as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106 (b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the outside wrapper or container and the immediate container the statement "Expiration date _____", the blank being filled in with the date that is 60 months, or 24 months if it is packaged in an immediate container other than tin or glass, or 12 months if the ointment base is water miscible, after the month during which the batch was certified.

(ii) If it contains one of the active ingredients specified in paragraph (a)

(1) of this section, after the name "chloramphenicol ointment", wherever it appears, the name of the active ingredient, in juxtaposition with such name.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The chloramphenicol used in making the batch for potency, pH, specific rotation, melting point, and absorptivity, and for toxicity if the ointment is intended for ophthalmic use.

(*b*) The batch for potency and for sterility if the ointment is intended for ophthalmic use.

(ii) Samples required:

(*a*) The chloramphenicol used in making the batch: 10 packages, each containing approximately 300 milligrams, packaged in accordance with the requirements of § 455.10b(a)(2).

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 5 immediate containers if it is packaged in immediate containers of tin or glass; a minimum of 20 immediate containers if it is packaged in immediate containers other than tin or glass.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows:

(i) *If the ointment is water miscible.* Place an accurately weighed representative portion of the sample into a high-speed glass blender jar containing 1.0 milliliter polysorbate 80 and sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 1 to the reference concentration of 2.5 micrograms of chloramphenicol per milliliter (estimated).

(ii) *If the ointment is not water miscible.* Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of petroleum ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 1 percent potassium phosphate buffer, pH 6.0 (solution 1), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of three more 20 to 25-milliliter quantities of solution 1. Combine the buffer extractives in a suitable volumetric flask and dilute to volume with solution 1. Remove an aliquot and further dilute with solution 1 to the reference concentration of 2.5 micrograms of chloramphenicol per milliliter (estimated). The potency of chloramphenicol ointment is satisfactory if it contains not less than 90 percent and not more than 130 percent of the number of milligrams of chloramphenicol that it is represented to contain.

(2) *Sterility.* If the ointment is intended for ophthalmic use, proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(3) of that section. However, if the ointment is not soluble in isopropyl myristate proceed as directed in § 436.20 of this chapter, using the method described in § 436.20(e)(2), except use 100 milligrams in lieu of 300 milligrams of solids.

§ 455.310d Chloramphenicol-polymyxin ointment.

(a) *Requirements for certification.* Chloramphenicol-polymyxin ointment conforms to all requirements and is subject to all procedures prescribed by § 455.310c(a) for chloramphenicol ointment, except that:

(1) It contains not less than 5,000 units of polymyxin B per gram. The polymyxin B used conforms to the requirements prescribed for polymyxin B by § 444.170a(a)(1) of this chapter.

(2) In lieu of the labeling prescribed by § 455.310c(a)(3)(i)(*a*), each package shall bear on the outside wrapper or container and the immediate container, the statement "Expiration date _____", the blank being filled in with the date that is 24 months after the month during which the batch was certified, except that the blank may be filled in with the date that is 36 months, 48 months, or 60 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time such drug as prepared by him complies with the standards prescribed by this section: *Provided however,* That such expiration date may be omitted from the immediate container if it contains a single dose and it is packaged in an individual wrapper or container.

(3) In addition to complying with the requirements of § 455.310c(a)(4), a person who requests certification of a batch shall submit with his request a statement showing the batch mark and (unless previously submitted) the results and date of the latest tests and assays of the polymyxin used in making the batch for potency and toxicity. He shall also submit in connection with his request a sample consisting of not less than 6 packages of ointment and (unless it was previously submitted) a sample consisting of 5 packages containing approximately equal portions of not less than 0.5 gram each of the polymyxin used in making the batch.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Chloramphenicol content.* Proceed as directed in § 455.310c(b). Its chloramphenicol content is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams per gram that it is represented to contain.

(ii) *Polymyxin content.* Proceed as directed in § 444.170a(b)(2)(i) of this chapter, except in lieu of the directions in § 444.170a(b)(2)(i)(*g*) of this chapter for the preparation of the sample, prepare the sample as follows: Place an accurately weighed sample (usually approximately 1.0 gram) in a separatory funnel containing approximately 50 milliliters of peroxide-free ether, and shake the sample and ether until homogeneous. Add 25 milliliters of 10-percent potassium phosphate buffer, pH 6.0 and shake. Remove the buffer layer and repeat the extraction with three additional 25-milliliter portions of buffer. Combine the extractives and make the proper estimated dilutions, using the buffer solution, except that, if the sample contains a water-soluble base, place an accurately weighed representative sample in a blending jar containing 1.0 milliliter of polysorbate 80 and sufficient 10 percent potassium phosphate buffer, pH 6.0, to give a final volume of 200 milliliters. Using a high-speed blender, blend the mixture for 2 minutes to 3 minutes and then make the proper estimated dilutions with 10 percent phosphate buffer pH 6.0. Its content of polymyxin is satisfactory if it contains not less than 90 percent and not more than 125 percent of the number of units per gram that it is represented to contain.

(2) *Sterility.* If the ointment is intended for ophthalmic use, proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(3) of that section. However, if the ointment is not soluble in isopropyl myristate proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except use 100 milligrams in lieu of 300 milligrams of solids.

Subpart E—Otic Dosage Forms

§ 455.410 Chloramphenicol otic; chloramphenicol topical.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chloramphenicol otic and chloramphenicol topical is chloramphenicol, with or without ethyl aminobenzoate, in a suitable and harmless vehicle. The potency of the solution is not less than 5 milligrams per milliliter. Its moisture content is not more than 2 percent. Its pH is not less than 4 and not more than 8. The chloramphenicol used conforms to the requirements of § 455.10b(a)(1), except paragraph (a)(1)(ii), (iii), (iv), and (v) of that section. Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* Each immediate container shall be a tight container as defined by the U.S.P. and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 1.106(b) of this chapter (regulations issued under section 502(f) of the act), each package shall bear on the outside wrapper or container and the immediate container the following:

(i) The statement "Expiration date _____", the blank being filled in with the date that is 12 months after the month during which the batch was certified, except that the blank may be filled in with the date that is 18, 24, or 30 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time such drug as prepared by him complies with the standards prescribed by paragraph (a)(1) of this section.

(ii) The statement "*Warning*—For external use only".

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch the batch mark, and (unless it was previously submitted) the date on which the latest assay of the chloramphenicol used in making the batch was completed, the number of milligrams of chloramphenicol in each milliliter of the batch, the date on which the latest assay of the drug comprising such batch was completed, the quantity of each other ingredient used in making the batch, and a statement that each such ingredient conforms to the requirements prescribed therefor by this section.

(ii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request results of the tests and assays listed after each of the following, made by him on an accurately representative sample of:

(*a*) The batch: potency, moisture, and pH.

(*b*) The chloramphenicol used in making the batch; potency, pH, specific rotation, melting point, and absorptivity.

(iii) Except as otherwise provided by paragraph (a)(4)(iv) of this section, such person shall submit in connection with his request, in the quantities hereinafter indicated, accurately representative samples of the following:

(*a*) The batch; one immediate container for each 5,000 immediate containers in the batch, but in no case less than 20 immediate containers, collected by taking single immediate containers, before or after labeling, at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(*b*) The chloramphenicol used in making the batch; 10 packages, each containing approximately equal portions of not less than 300 milligrams, packaged in accordance with the requirements of § 455.10b(a)(2).

(*c*) In case of an initial request for certification, each other ingredient used in making the batch; one package of each containing approximately 5.0 grams.

(iv) No result referred to in paragraph (a)(4)(ii)(*b*) of this section, and no sample referred to in paragraph (a)(4)(iii)(*b*) of this section, is required if such result or sample has been previously submitted.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative aliquot of the sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a convenient concentration. Further dilute with solution 1 to the reference concentration of 2.5 micrograms of chloramphenicol per milliliter (estimated). Its potency is satisfactory if it contains not less than 90 percent and not more than 130 percent of the number of milligrams it is represented to contain.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(c) *pH.* Proceed as directed in § 436.202 of this chapter, using the sample diluted with an equal volume of distilled water.

Subpart F—Dermatologic Dosage Forms

§ 455.503a Calcium amphomycin-neomycin sulfate-hydrocortisone acetate cream.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Calcium amphomycin-neomycin sulfate-hydrocortisone acetate cream is calcium amphomycin, neomycin sulfate, and hydrocortisone acetate in a suitable and harmless water-soluble cream base containing suitable buffers, emulsifiers, and preservatives. Each gram contains 5 milligrams of amphomycin, 3.3 milligrams of neomycin, and 10 milligrams of hydrocortisone acetate. Its amphomycin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of amphomycin that it is represented to contain. Its neomycin content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of neomycin that it is represented to contain. The calcium amphomycin used in making the cream conforms to the standards prescribed by § 455.3(a)(1). The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1)(i), (v), (vi), and (vii) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The calcium amphomycin used in making the batch for potency, moisture, and pH.

(*b*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity.

(*c*) The batch for amphomycin potency and neomycin sulfate potency.

(ii) Samples required:

(*a*) Calcium amphomycin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) Neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The batch: A minimum of 6 immediate containers.

(b) *Tests and methods of assay*—(1) *Amphomycin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Accurately weigh approximately 2.0 grams of the sample and place in a high-speed blender, add 1.0 milliliter of polysorbate 80 and sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a final volume of 200 milliliters. Blend for 2 minutes. Further dilute with solution 3 to the reference concentration of 10 micrograms of amphomycin per milliliter (estimated).

(2) *Neomycin content.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Blend the sample as described in paragraph (b)(1) of this section and further dilute with solution 3 to the reference concentration of 1 microgram of neomycin per milliliter (estimated).

§ 455.510 Chloramphenicol dermatologic dosage forms.

§ 455.510a Chloramphenicol ointment (chloramphenicol cream).

The requirements for certification and the tests and methods of assay for chloramphenicol ointment (chloramphenicol cream) are described in § 455.310c.

§ 455.510b Chloramphenicol otic; chloramphenicol topical.

The requirements for certification and the tests and methods of assay for chloramphenicol otic; chloramphenicol topical are described in § 455.410.

§ 455.510c Chloramphenicol-polymyxin ointment.

The requirements for certification and the tests and methods of assay for chloramphenicol-polymyxin ointment are described in § 455.310d.

PART 460—ANTIBIOTIC DRUGS INTENDED FOR USE IN LABORATORY DIAGNOSIS OF DISEASE

Subpart A—Susceptibility Discs

Sec.
460.1 Certification procedure for antibiotic sensitivity discs.
460.6 Tests and methods of assay for potency of antibiotic susceptibility discs.
460.15 Streptomycin sulfate discs for use in culture media.

Subpart B—Susceptibility Powders

460.25 Bacitracin diagnostic sensitivity powder.
460.28 Disodium carbenicillin diagnostic sensitivity powder.
460.33 Clindamycin hydrochloride hydrate sensitivity powder.
460.38 Sodium colistimethate diagnostic sensitivity powder.
460.42 Dihydrostreptomycin sulfate diagnostic sensitivity powder.
460.47 Doxycycline hyclate diagnostic sensitivity powder.
460.55 Lincomycin hydrochloride monohydrate diagnostic sensitivity powder.
460.58 Methacycline hydrochloride diagnostic sensitivity powder.

Sec.
460.64 Minocycline hydrochloride powder for microbial susceptibility testing.
460.66 Oleandomycin phosphate diagnostic sensitivity powder.
460.70 Oxytetracycline hydrochloride diagnostic sensitivity powder.
460.75 Potassium penicillin G diagnostic sensitivity powder.
460.79 Polymyxin B sulfate diagnostic sensitivity powder.
460.86 Spectinomycin hydrochloride powder for microbial susceptibility testing.
460.89 Streptomycin sulfate diagnostic sensitivity powder.
460.93 Tetracycline hydrochloride diagnostic sensitivity powder.

Subpart A—Susceptibility Discs

§ 460.1 Certification procedure for antibiotic sensitivity discs.

(a) *Standards of identity, strength, quality, and purity.* Antibiotic susceptibility discs are round flat discs that have a diameter of one-fourth inch and are made of clear absorbent paper containing antibiotic compounds. They are capable of absorbing moisture rapidly and the antibiotic is evenly distributed. The thickness is sufficient to assure rigidity and to have permitted the complete absorption of an adequate volume of antibiotic solution (approximately 0.02 milliliter). The identity of each disc is signified either by a color or by means of an identifying sign. The absorbent paper and dye or ink used must not affect either bacterial growth or the antibiotic. Each disc shall have a uniform potency that is equivalent to that contained in a standard disc prepared with one of the following quantities of antibiotic drugs:

Ampicillin: 10 mcg.
Bacitracin: 10 units.
Carbenicillin: 50 mcg.
Cephalothin: 30 mcg.
Chloramphenicol: 30 mcg.
Clindamycin: 2 mcg.
Colistin: 10 mcg.
Erythromycin: 15 mcg.
Gentamicin: 10 mcg.
Kanamycin: 30 mcg.
Methicillin: 5 mcg.
Neomycin: 30 mcg.
Novobiocin: 30 mcg.
Oleandomycin: 15 mcg.
Penicillin G: 10 units.
Polymyxin B: 300 units.
Rifampin: 5 mcg.
Streptomycin: 10 mcg.
Tetracycline: 30 mcg.
Vancomycin: 30 mcg.

The standard discs used to determine the potency shall be made of paper as described in § 460.6(d). Each antibiotic compound used to impregnate such standard discs shall be equilibrated in terms of the working standard designated by the Commissioner for use in determining the potency or purity of such antibiotic.

(b) *Packaging.* The immediate container shall be a tight container as defined by the U.S.P. and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused that are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded. Each immediate container may contain a desiccant, and each may be packaged in combination with containers of suitable discs of drugs other than those described in paragraph (a) of this section. Such other discs shall be suitable only if the manufacturer and packer have submitted to the Commissioner information of the kind described in § 431.17 of this chapter, and such information has been accepted by the Commissioner.

(c) *Labeling.* Each package of discs shall bear on its label or labeling, as hereinafter indicated, the following:

(1) On the outside wrapper or container and the immediate container:

(i) The batch mark.

(ii) The name and potency of each disc in the batch according to the following:

Name of Disc	*Content of antibiotic in micrograms or units per disc*
Ampicillin-class disc.	10 mcg. ampicillin.
Bacitracin disc	10 units bacitracin.
Carbenicillin disc.	50 mcg. carbenicillin.
Cephalosporin-class disc.	30 mcg. cephalothin.
Chloramphenicol disc.	30 mcg. chloramphenicol.
Colistin disc	10 mcg. colistin.
Erythromycin disc.	15 mcg. erythromycin.
Gentamicin disc.	10 mcg. gentamicin.
Kanamycin disc	30 mcg. kanamycin.
Lincomycin-class disc.	2 mcg. clindamycin.
Neomycin disc	30 mcg. neomycin.
Novobiocin disc	30 mcg. novobiocin.
Oleandomycin disc.	15 mcg. oleandomycin.
Penicillin-class disc.	10 units penicillin G.
Penicillinase-resistant penicillin-class disc.	5 mcg. methicillin.
Polymyxin B disc	300 units polymyxin B.
Rifampin disc	5 mcg. rifampin.
Streptomycin-class disc.	10 mcg. streptomycin.
Tetracycline-class disc.	30 mcg. tetracycline.
Vancomycin disc.	30 mcg. vancomycin.

(iii) The statement "Expiration date _____", the blank being filled in with the date that is 6 months after the month during which the batch was certified, except that the blank may be filled in with a date that is 12, 18, 24, 30, 36, 42, 48, 54, or 60 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that such drug as prepared by him is stable for such longer period of time. If it is a packaged combination of discs of two or more drugs, its outside wrapper shall bear only one expiration date, and that date shall be the date that is required for the shortest dated discs contained in the package.

(iv) The statement "For laboratory use only".

(2) On the circular or other labeling within or attached to the package, adequate directions for the use of such discs, including the following recommended method:

STANDARDIZED DISC SUSCEPTIBILITY TEST

DIRECTIONS FOR USE

Quantitative methods that require the measurement of zone sizes give the most precise estimates of antibiotic susceptibilities. The following outline describes such a procedure. Minor variations from this procedure may be used if the resulting procedure is standardized according to the results obtained in the laboratory from adequate studies with control cultures.

A. PREPARATION OF CULTURE MEDIUM AND PLATES

1. Melt previously prepared and sterilized Mueller-Hinton agar medium and cool to 45°–50° C.

2. For the purpose of testing certain fastidious organisms such as streptococci and *Haemophilus* species, 5 percent defibrinated human, horse, or sheep blood may be added to the above medium which is "chocolatized" when indicated.

3. To prepare the plates, pour the melted medium into Petri dishes on a level surface to a depth of 4 millimeters.

4. Let the medium harden and allow to stand long enough for excess moisture to evaporate. (For this purpose plates may be placed in an incubator at 35°–37° C. for 15–30 minutes or allowed to stand somewhat longer at room temperature.) There should be no moisture droplets on the surface of the medium or on the petri dish covers. The pH of the solidified medium should be 7.2–7.4. Satisfactory plates may be used immediately or refrigerated. Plates may be used as long as the surface is moist and there is no sign of deterioration.

NOTE: Commercially prepared agar plates meeting the above specifications may be used.

B. PREPARATION OF INOCULUM

1. Select four or five similar colonies.

2. Transfer these colonies (obtained by touching the top of each colony in turn with a wire loop) in turn to a test tube containing about 5 milliliters of a suitable liquid medium such as soybean-casein digest broth, U.S.P.

3. Incubate the tube at 35°–37° C. long enough (2 to 8 hours) to produce an organism suspension with moderate cloudiness. At that point the inoculum density of the suspension should be controlled by diluting it, or a portion of it, with sterile saline to obtain a turbidity equivalent to that of a freshly prepared turbidity standard obtained by adding 0.5 milliliter of 1.175 percent barium chloride dihydrate ($BaCl_2 2H_2O$) solution to 99.5 milliliters of 0.36 N (1.0 percent)

sulfuric acid. Other suitable methods for standardizing inoculum density may be used; for example, a photometric method. In some cases it may be possible to get an adequate inoculum density in the tube even without incubation.

NOTE: Extremes in inoculum density should be avoided. Undiluted overnight broth culture should never be used for streaking plates.

C. INOCULATING THE PLATES

1. Dip a sterile cotton swab on a wooden applicator into the properly diluted inoculum. Remove excess inoculum from the swab by rotating it several times with firm pressure on the inside wall of the test tube above the fluid level.

2. Streak the swab over the entire sterile agar surface of a plate. Streaking successively in three different directions is recommended to obtain an even inoculum.

3. Replace the plate top and allow the inoculum to dry for 3 to 5 minutes.

4. Place the susceptibility discs on the inoculated agar surface and with sterile forceps, or needle tip flamed and cooled between each use, gently press down each disc to insure even contact. Space the discs evenly so that they are no closer than 10 to 15 millimeters to the edge of the petri dish and sufficiently separated from each other to avoid overlapping zones of inhibition. (Spacing may be accomplished by using a disc dispenser or by putting the plate over a pattern to guide the placement of discs.) Within 30 minutes, place the plate in an incubator under aerobic conditions at a constant temperature in the range of 35°–37° C.

5. Read the plate after overnight incubation or, if rapid results are desired, the diameters of the zone of inhibition may be readable after 6 to 8 hours incubation. In the latter case, the results should be confirmed by also reading the results after overnight incubation.

NOTE: Microbial growth on the plate should be just or almost confluent. If only isolated colonies are present the inoculum was too light and the test should be repeated.

Modifications of the inoculation procedure described in 1–3 above, such as the use of the agar overlay method described in Barry, A. L., Garcia, F., and Thrupp, L. D.: "An Improved Single-disk Method for Testing the Antibiotic Susceptibility of Rapidly-growing Pathogens." Amer. J. Clin. Pathol. 53:149–58, 1970,[12] a copy of which is on file with the Office of the Federal Register, may be used if the procedure is standardized to produce results with the control cultures that are equivalent to those obtained with the recommended cotton swab streak method.

D. READING THE PLATES

Measure and record the diameter of each zone (including the diameter of the disc) to the closest millimeter, reading to the point of complete inhibition as judged by the unaided eye. Preferably, read from the underside of the plate without removing the cover, using a ruler, calipers, transparent plastic gage, or other device. A mechanical zone reader may be used. If blood agar is used, measure the zones from the surface with the cover removed from the plate.

E. INTERPRETATION OF ZONE SIZES

Interpret the susceptibility according to the following table:

Antibiotic	Disc content	Diameter (millimeters) of zone of inhibition		
		Resistant	Intermediate	Susceptible
Ampicillin[1] when testing gram-negative micro-organisms and enterococci.	10 mcg	11 or less	12–13	14 or more.
Ampicillin[1] when testing staphylococci and penicillin G—susceptible micro-organisms.	10 mcg	20 or less	21–28	29 or more.
Ampicillin[1] when testing *Haemophilus* species	10 mcg	19 or less		20 or more.
Bacitracin	10 units	8 or less	9–12	13 or more.
Carbenicillin when testing *Proteus* species and *Escherichia coli*.	50 mcg	17 or less	18–22	23 or more.
Carbenicillin when testing *Pseudomonas aeruginosa*	50 mcg	12 or less	13–14	15 or more.
Cephalothin when reporting susceptibility to cephalothin, cephaloridine, and cephalexin.	30 mcg	14 or less	15–17	18 or more.[2]
Cephalothin when reporting susceptibility to cephaloglycin.	30 mcg	14 or less		15 or more.
Chloramphenicol	30 mcg	12 or less	13–17	18 or more.
Clindamycin[3] when reporting susceptibility to clindamycin.	2 mcg	14 or less	15–16	17 or more.
Clindamycin when reporting susceptibility to lincomycin.	2 mcg	16 or less	17–20	21 or more.
Colistin	10 mcg	8 or less	9–10	11 or more.[4]
Erythromycin	15 mcg	13 or less	14–17	18 or more.
Gentamicin	10 mcg	12 or less		13 or more.
Kanamycin	30 mcg	13 or less	14–17	18 or more.
Methicillin[5]	5 mcg	9 or less	10–13	14 or more.
Neomycin	30 mcg	12 or less	13–16	17 or more.
Novobiocin	30 mcg	17 or less	18–21	22 or more.[6]
Oleandomycin[7]	15 mcg	11 or less	12–16	17 or more.
Penicillin G when testing staphylococci[8]	10 units	20 or less	21–28	29 or more.
Penicillin G when testing other micro-organisms[9]	10 units	11 or less	12–21	22 or more.
Polymyxin B	300 units	8 or less	9–11	12 or more.[4]
Rifampin when testing *Neisseria meningitidis* susceptibility only.	5 mcg	24 or less		25 or more.
Streptomycin	10 mcg	11 or less	12–14	15 or more.
Tetracycline[10]	30 mcg	14 or less	15–18	19 or more.
Vancomycin	30 mcg	9 or less	10–11	12 or more.

[1] The ampicillin disc is used for testing susceptibility to both ampicillin and hetacillin.

[2] Staphylococci exhibiting resistance to the penicillinase-resistant penicillin class discs should be reported as resistant to cephalosporin class antibiotics. The 30 mcg. cephalothin disc cannot be relied upon to detect resistance of methicillin-resistant staphylococci to cephalosporin class antibiotics.

[3] The clindamycin disc is used for testing susceptibility to both clindamycin and lincomycin.

[4] Colistin and polymyxin B diffuse poorly in agar and the accuracy of the diffusion method is thus less than with other antibiotics. Resistance is always significant, but when treatment of systemic infections due to susceptible strains is considered, it is wise to confirm the results of a diffusion test with a dilution method.

[5] The methicillin disc is used for testing susceptibility to all penicillinase-resistant penicillins; that is, methicillin, cloxacillin, dicloxacillin, oxacillin, and nafcillin.

[6] Not applicable to medium that contains blood.

[7] The oleandomycin disc is used for testing susceptibility to oleandomycin and troleandomycin.

[8] The penicillin G disc is used for testing susceptibility to all penicillinase-susceptible penicillins except ampicillin and carbenicillin; that is, penicillin G, phenoxymethyl penicillin, and phenethicillin.

[9] This category includes some organisms such as enterococci and gram-negative bacilli that may cause systemic infections treatable with high doses of penicillin G. Such organisms should only be reported susceptible to penicillin G and not to phenoxymethyl penicillin or phenethicillin.

[10] The tetracycline disc is used for testing susceptibility to all tetracyclines; that is, chlortetracycline, demeclocycline, doxycycline, methacycline, oxytetracycline, rolitetracycline, minocycline, and tetracycline.

F. REFERENCE ORGANISMS

1. Maintain stock cultures of *Staphylococcus aureus* (ATCC 25923)[1] and *Escherichia coli* (ATCC 25922).[1]

2. Test these reference organisms daily by the above procedure using antibiotic discs representative of those to be used in the testing of clinical isolates.

3. The individual values of zone sizes for the control organisms can be expected to fall in the ranges indicated in the following table:

Antibiotic	Disc content	Individual tests		
		Zone diameter in millimeters		Permitted millimeter difference ATCC 25923–ATCC 25922
		With *S. aureus* ATCC 25923	With *E. coli* ATCC 25922	
Ampicillin	10 mcg	24–35	15–20	7–17
Bacitracin	10 units	17–22		
Cephalothin	30 mcg	25–37	18–23	5–16
Chloramphenicol	30 mcg	19–26	21–27	−4–1
Clindamycin	2 mcg	19–29		
Colistin	10 mcg		11–15	
Erythromycin	15 mcg	22–30	8–14	10–19
Gentamicin	10 mcg	19–27	19–26	−2–3
Kanamycin	30 mcg	19–26	17–25	−1–4
Methicillin	5 mcg	17–22		
Neomycin	30 mcg	15–21	17–23	0–3
Novobiocin	30 mcg	22–31		
Oleandomycin	15 mcg	19–28		
Penicillin G	10 units	26–37		
Polymyxin B	300 units	7–13	13–16	−7–−2
Streptomycin	10 mcg	14–22	19–20	−1–5
Tetracycline	30 mcg	19–28	18–25	0–6
Vancomycin	30 mcg	15–19		

[12] Copies may be obtained from: J. B. Lippincott Company, Attn: Circulation Manager, East Washington Square, Philadelphia, PA 19105.

6. LIMITATIONS OF THE METHOD

The method of interpretation described in E above applies to rapidly growing pathogens and should not be applied to slowly growing organisms. The latter show larger zones of inhibition than those given in the table. Susceptibility of gonococci to penicillin, and of slow-growing strains, e.g., *Bacteroides* species and fastidious anaerobes to any antibiotic, should be determined by the broth-dilution or agar-dilution method unless specifically standardized diffusion tests are used.

§ 460.6 Tests and methods of assay for potency of antibiotic susceptibility discs.

(a) *Culture media.* Use ingredients that conform to the standards prescribed by the United States Pharmacopeia or The National Formulary. In lieu of preparing the media from the individual ingredients, they may be made from a dehydrated mixture which, when reconstituted with distilled water, has the same composition as such media. Minor modification of the specified individual ingredients is permissible if the resulting media possess growth-promoting properties at least equal to the media described.

(1) *Medium A:*

Peptone	6.0 gm.
Pancreatic digest of casein	4.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Dextrose	1.0 gm.
Agar	15.0 gm.
Distilled water, q. s.	1,000.0 ml.

pH 6.5 to 6.6 after sterilization.

(2) *Medium B.* Same as medium A, except that it also contains 300 milligrams of hydrated manganese sulfate per liter.

(3) *Medium C.* Same as medium A, except that the final pH is adjusted from 7.9 to 8.1 after sterilization.

(4) *Medium D:*

Peptone	5.0 gm.
Yeast extract	1.5 gm.
Beef extract	1.5 gm.
Sodium chloride	3.5 gm.
Dextrose	1.0 gm.
Dipotassium phosphate	3.68 gm.
Potassium dihydrogen phosphate	1.32 gm.
Distilled water, q.s.	1,000.0 ml.

pH 7.0 after sterilization.

(5) *Medium E:*

Peptone	6.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 6.5 to 6.6 after sterilization.

(6) *Medium F:*

Pancreatic digest of casein	17.0 gm.
Papaic digest of soybean	3.0 gm.
Sodium chloride	5.0 gm.
Dipotassium phosphate	2.5 gm.
Dextrose	2.5 gm.
Agar	20.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 7.3 after sterilization.

(7) *Medium G.* Same as medium F, except for the following:

Agar	12.0 gm.
Polysorbate 80 (sterile)	10.0 gm.

Add polysorbate 80 after boiling.

(8) *Medium H:*

Peptone	9.4 gm.
Yeast extract	4.7 gm.
Beef extract	2.4 gm.
Sodium chloride	10.0 gm.
Dextrose	10.0 gm.
Agar	28.5 gm.
Distilled water, q.s.	1,000.0 ml.

pH 6.0 to 6.5 after sterilization.

(9) *Medium I:*

Peptone	6.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Dextrose	1.0 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000.0 ml.

6.6 after sterilization.

(10) *Medium J:*

Pancreatic digest of casein	15.0 gm.
Papaic digest of soybean	5.0 gm.
Sodium chloride	5.0 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 7.3 after sterilization.

(11) *Medium K:*

Pancreatic digest of casein	17.0 gm.
Papaic digest of soybean	3.0 gm.
Sodium chloride	5.0 gm.
Dipotassium phosphate	2.5 gm.
Dextrose	2.5 gm.
Distilled water, q.s.	1,000.0 ml.

pH 7.3 after sterilization.

(b) *Preparation of test organism suspensions*—(1) *Suspension 1. Staphylococcus aureus* (ATCC 6538P)[1] is maintained and grown on medium A. Wash the organisms from an agar slant, incubated for 24 hours at 32° C. to 35° C., with 3.0 milliliters of sterile sodium chloride solution onto the agar surface of a Roux bottle containing 300 milliliters of medium A. Spread the suspension of organisms over the entire agar surface with the aid of sterile glass beads. Incubate 24 hours at 32° C. to 35° C. Wash the resulting growth from the agar surface with about 50 milliliters of sterile sodium chloride solution. Standardize this stock suspension by determining the dilution that will permit 20 percent light transmission. Store the stock suspension in the refrigerator (1 week) and use the indicated dilution prepared daily.

(2) *Suspension 2.* Follow the procedure described for suspension 1, except standardize the bulk suspension so that a 1:10 dilution in saline solution gives 20 percent light transmission. In this case, the bulk suspension, and not the 1:10 dilution of it, is used for the inoculum.

(3) *Suspension 3.* The test organism is *Staphylococcus aureus* (ATCC 13150).[1] Follow the procedure described for suspension 1, but determine how much the bulk suspension should be diluted to obtain a suspension permitting 80 percent light transmission. Use the indicated dilution prepared daily for the inoculum for the plates.

(4) *Suspension 4. Sarcina lutea* (ATCC 9341)[1] is maintained on agar slants of medium A and transferred to fresh slants approximately every 2 weeks. This culture is incubated overnight at 26° C., and then stored in the refrigerator. Prepare an inoculum for the plates as follows: Streak an agar slant heavily with the test organism and incubate for 24 hours at 26° C. Wash the growth from the slant with 3 milliliters to 4 milliliters of medium D, and transfer to the surface of a Roux bottle containing 300 milliliters of medium A. Spread the suspension evenly over the entire surface with the aid of sterile glass beads. Incubate for 24 hours at 26° C. Wash the growth from the agar surface with 15 milliliters of medium D. If an aliquot of this bulk suspension when diluted 1:10 with medium D gives 10 percent light transmission, the bulk suspension is satisfactory for use. It may be necessary to adjust the bulk suspension by dilution so that an aliquot of the adjusted suspension when diluted 1:10 will give the desired 10 percent light transmission. The adjusted bulk suspension only, and not the 1:10 dilution of it, is used in preparing the inoculum. Store the stock suspension in the refrigerator and use for 2 weeks.

(5) *Suspension 5. Bacillus subtilis* (ATCC 6633)[1] is maintained on agar medium A and transferred to a fresh slant every month. To prepare the spore suspension, inoculate a fresh slant of agar medium A with the test organism and incubate at 37° C. for 16 hours to 24 hours. Wash the culture from the slant with 3 milliliters of sterile sodium chloride solution onto the surface of a Roux bottle containing 300 milliliters of agar medium B. Incubate for 5 days at 37° C. Suspend the growth in 50 milliliters of sterile saline solution, centrifuge, and decant the supernatant liquid. Reconstitute the sediment and heat-shock the suspension by heating for 30 minutes at 70° C. Store the spore suspension in the refrigerator. It may be kept several months. Light transmission is not used for standardization.

(6) *Suspension 6. Staphylococcus epidermidis* (ATCC 12228)[1] is maintained on medium A and transferred to a fresh slant once a week. Inoculate a fresh slant of medium A with the test organism and incubate at 32° C. to 35° C. for 24 hours. Wash the culture from the slant with 3 milliliters of sterile sodium chloride solution onto the surface of a Roux bottle containing 300 milliliters of medium A. Incubate at 32° C. to 35° C. for 24 hours. Wash the resulting growth from the agar surface with about 50 milliliters of sterile sodium chloride solution. Standardize this stock suspension by determining the dilution that will give 80 percent light transmission. Store the stock suspension in the refrigerator (1 week) and use the indicated dilution prepared daily for the inoculum for the plates.

(7) *Suspension 7. Bordetella bronchiseptica* (ATCC 4617)[1] is maintained on medium F and transferred to a fresh slant every 2 weeks. To prepare a stock suspension inoculate a fresh slant of medium F and incubate at 37° C. for 16 hours to 24 hours. Wash the culture

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

from this slant with 3 milliliters of sterile distilled water onto the surface of a Roux bottle containing 300 milliliters of medium F, and incubate 24 hours at 37° C. Wash off the growth with 50 milliliters of sterile distilled water and standardize the resulting stock suspension by determining the dilution that will give 50 percent light transmission. Store the stock suspension in the refrigerator (2 weeks), and use the indicated dilution prepared daily for the inoculum for the plates.

(8) *Suspension 8. Saccharomyces cerevisiae* (ATCC 9763)[1] is maintained on slants of medium H and transferred once a week. After transfer, the culture is incubated at 37° C. for 24 hours and then kept refrigerated. Wash the organism from a freshly incubated agar slant with 3 milliliters of sterile saline solution onto the agar surface in a Roux bottle containing 300 milliliters of medium H. Spread the suspension of organisms over the entire agar surface with the aid of sterile glass beads. Incubate for 24 hours at 37° C. and then wash the resulting growth from the agar surface with about 25 milliliters of sterile saline solution. Store the suspension in the refrigerator and use for 1 month.

(9) *Suspension 9.* Follow the procedure described for suspension 1, except determine how much the bulk suspension should be diluted to obtain a suspension permitting 80 percent light transmission. Use the indicated dilution, prepared daily, for the inoculum for the plates.

(10) *Suspension 10; Klebsiella pneumoniae* (ATCC 10031),[1] noncapsulated, is maintained on medium A and transferred to a fresh slant once a week. Inoculate a fresh slant of medium A with the test organism and incubate overnight at 32° C.–35° C. Wash the culture from the slant with 3 milliliters of sterilized U.S.P. saline T.S. onto the surface of a Roux bottle containing 300 milliliters of medium A. Incubate at 32° C.–35° C. for 24 hours. Wash the resulting growth from the agar surface with about 50 milliliters of sterilized U.S.P. saline T.S. If an aliquot of this bulk suspension when diluted 1:9 with saline solution gives 40 percent light transmission, the bulk suspension is satisfactory for use. It may be necessary to adjust the bulk suspension by dilution so that an aliquot of the adjusted suspension when diluted 1:9 will give the desired 40 percent light transmission. The adjusted bulk suspension (not the 1:9 dilution) is used in preparing the inoculum. Store the suspension in the refrigerator and use for no more than 1 week.

(11) *Suspension 11. Streptococcus fecalis* (ATCC 14506)[1] is maintained on medium E and transferred to a fresh agar slant once a week. After transfer, the culture is incubated at 37° C. for 24 hours and then kept refrigerated. Transfer from a freshly incubated agar slant to a tube containing 10 milliliters of culture medium described in § 147.3 (b)(1). Incubate the broth culture for 16 to 18 hours at 37° C. and store in the refrigerator. This culture may be used for no more than 1 week.

The light transmission values referred to in this paragraph were determined with a Lumetron Model 400–A photoelectric colorimeter at a wavelength of 650 millimicrons. If other instruments are used, different light transmission readings will probably be obtained. The values given are to be used as guides in this paragraph.

(12) *Suspension 12. Pseudomonas aeruginosa* (ATCC 25619)[1] is maintained and grown on medium J and transferred to a fresh agar slant once a week. Inoculate a fresh slant of medium J with the test organism and incubate at 37° C. for 24 hours. Transfer the culture from this slant with sterile glass beads onto the agar surface of a Roux bottle containing 300 milliliters of medium J. Spread the organisms over the entire agar surface with the aid of the glass beads. Incubate 24 hours at 37° C. Wash the resulting growth from the agar surface with about 30 milliliters of medium K. Do not standardize the suspension. Store the stock suspension under refrigeration and use for 2 weeks.

(c) *Preparation of plates*—(1) *Base layer.* Depending on the particular antibiotic in the discs to be tested, add 42 milliliters of the appropriate medium prescribed in paragraph (c)(3) of this section to each Petri dish (20 millimeters x 150 millimeters) and allow to harden on a flat, level surface and dry slightly by raising the tops on one side.

(2) *Seed layer.* Add the appropriate amount of inoculum, as prescribed by paragraph (c)(3) of this section, to the seed agar which has been melted and cooled to 48° C. Swirl the flasks to obtain a homogeneous suspension. Add 8 milliliters of the appropriate seed agar, as specified in paragraph (c)(3) of this section, to each plate, spread evenly over the hardened base layer, and allow to harden and dry on a flat level surface. For accurate results, it is necessary to obtain uniform distribution of the agar over the surface of the plates.

(3) *Inoculum and media to be used.* Depending on the particular antibiotic in the disc to be tested, select from the following table the inoculum and media to be used:

Antibiotic	Volume of suspension added to each 100 ml. of seed agar used for test	Suspension number	Medium	
			Base layer	Seed layer
	Ml.			
Ampicillin	1.0	3	E	A
Bacitracin	1.0	3	E	A
Carbenicillin	3.0	12	F	G
Cephalogiycin (dihydrate)	1.0	10	E	A
Cephaloridine	1.0	10	E	A
Cephalothin	1.0	10	E	A
Chloramphenicol	4.0	4	E	A
Clindamycin	2.0	2	A	I
Colistin (sulfate)	1.0	7	F	G
Erythromycin	2.0	11	O	C
Gentamicin (sulfate)	0.5	3	O	C
Kanamycin (sulfate)	1.0	9	E	A
Methicillin	1.0	3	E	A
Neomycin (sulfate)	2.5	6	E	O
Novobiocin (sodium)	4.0	5	E	A
Oleandomycin (phosphate)	3.0	3	E	A
Penicillin G	1.0	3	E	A
Polymyxin B (sulfate)	1.0	7	F	G
Rifampin	1.0	5	E	A
Streptomycin (sulfate)	3.0	1	E	O
Tetracycline (hydrochloride)	1.5	4	E	A
Vancomycin	1.0	6	C	C

(d) *Preparation of control discs.* Use round, blank discs having a diameter of ¼-inch made of clear-white paper weighing 30 milligrams ±4 milligrams per square centimeter, and which will absorb 2.5 to 3.0 times its own weight of distilled water. The paper shall contain no material that either enhances or inhibits the activity of any antibacterial agent impregnated thereon. In addition, the paper shall contain no materials which will affect the pH of any solvent placed on it or buffer any solution placed on it. The following methods shall be used to determine the suitability in this regard of any paper proposed for this use: Weigh 2 grams of paper or paper discs into a clean, glass-stoppered, 250-milliliter flask. Add 30 milliliters of freshly boiled and cooled distilled water (the pH of which has been determined). Stopper and shake vigorously for 1 hour on a shaking machine. Filter through a medium-porosity sintered glass filter. Determine the pH of the filtrate. Take the two 10-milliliter aliquots. To one add 0.05 milliliter of 0.01*N* HCl. To the second aliquot add 0.05 milliliter of 0.01*N* NaOH. Determine the pH of each solution. The paper shall be satisfactory for use, if (1) the pH of the paper filtrate was not more than ±0.3 pH units different from the pH of the distilled water used; (2) the pH of the acidified aliquot was lowered by at least 1.0 pH units; (3) the pH of the alkalinized aliquot was raised by at least 1.5 pH units. Place blank discs on aluminum or stainless steel wire mesh which is supported in a manner to allow circu-

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

lation of air above and below the discs. Prepare the desired number of discs for each point on the standard curve by accurately adding 0.02-milliliter-increments of the appropriate standard stock solution to each disc, using a suitable pipette. Dry discs in circulating air or under vacuum. Discs may be stored for 2 weeks in a desiccator under refrigeration. Depending on the antibiotic contained in the sample to be tested, prepare the stock solutions for the standard discs by dissolving an accurately weighed quantity of the working standard in the solvent indicated to obtain stock solutions that will contain the following concentrations required for the standard discs:

Antibiotic	Solvent	Standard curve (antibiotic concentration per disc)
Ampicillin	Water	1.3, 2.4, 4.4, 8.1, 15.0 μg.
Bacitracin	do	1.3, 2.4, 4.4, 8.1, 15.0 units.
Carbenicillin	Methyl alcohol	25.0, 35.8, 50.0, 70.7, 100 μg.
Cephalothin	50 percent methyl alcohol	15.0, 21.2, 300, 42.4, 60.0 μg.
Chloramphenicol	do	3.3, 6.3, 12.2, 23.4, 45.0 μg.
Clindamycin	50 percent methyl alcohol	1.0, 1.41, 2.0, 2.82, 4.0 μg.
Colistin (sulfate)	Water	1.3, 2.4, 4.4, 8.1, 15.0 μg.
Erythromycin	Methylalcohol	1.3, 2.7, 5.4, 11.0, 22.0 μg.
Gentamicin (sulfate)	Water	5, 7.1, 10, 14.1, 20 μg.
Kanamycin (sulfate)	do	3.3, 6.3, 12.2, 23.4, 45 μg.
Methicillin	Water	1.3, 2.4, 4.4, 8.1, 15.0 μg.
Neomycin (sulfate)	do	1.3, 6.3, 12.2, 23.4, 45.0 μg.
Novobiocin (sodium)	do	3.3, 6.3, 12.2, 23.4, 45.0 μg.
Oleandomycin (phosphate)	Water	1.3, 2.7, 5.4, 11.0, 22.5 μg.
Penicillin G	do	1.3, 2.4, 4.4, 8.1, 15.0 units.
Polymyxin B (sulfate)	do	31, 43, 122, 234, 450 units.
Rifampin	Methyl alcohol	3.0, 6.0, 12.0, 24.0, 48.0 μg.
Streptomycin (sulfate)	Water	1.3, 2.4, 4.4, 8.1, 15.0 μg.
Tetracycline (hydrochloride)	Methyl alcohol	3.3, 6.3, 12.2, 23.4, 45.0 μg.
Vancomycin (hydrochloride)	Water	2.3, 6.3, 12.2, 23.4, 45.0 μg.

(e) *Assay*—(1) *Individual discs one-fourth inch in diameter.* On each of three plates prepared as directed in paragraph (c) of this section, place the five control discs for the standard curve and two discs from each batch to be tested. The control discs for the standard curve and the sample discs are placed on the plates in a random arrangement, with no discs being closer than 24 millimeters (on centers) to any other disc. Discs are placed on the plates with the aid of forceps within as short a period of time as possible (not to exceed 3 minutes per plate) and tapped gently to ensure an even seal. Incubate the plates overnight at 32° C.–35° C., except if it is cephalothin, colistin, or novobiocin, or polymyxin, or viomycin, the incubation temperature is 37° C. After incubation, measure the diameter of each circle of inhibition, using calipers or a measuring device of comparable accuracy. Average the three zone sizes for each of the five standard-curve concentrations and plot the mean sizes on the arithmetic scale of semilogarithmic graph paper with the antibiotic concentrations on the logarithmic scale. Use the following equation to calculate the best straight line:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where

L = the calculated zone size of the low concentration;

H = the calculated zone size of the high concentration;

a, b, c, d, e = the observed average zone sizes for each respective concentration, a being that for the lowest concentration.

Plot the values obtained for L and H and connect these two points with a straight line. Average the six sample zone sizes and read the corresponding antibiotic concentration of this mean from the standard curve. This is the potency obtained for a single assay. Perform two or more replicate assays on each of 2 days. The average of all assays is the potency of the sample disc.

(2) *Discs one-fourth inch in diameter attached to rings, spokes, or other devices.* Remove or cut the disc from the device, including a small portion of the device to which it is attached, before testing, and proceed as directed in paragraph (e) (1) of this section.

(3) *Individual discs with diameters larger than one-fourth inch but no larger than three-eighths inch for use in impregnating culture media.* Proceed as directed in paragraph (e) (1) of this section, except instead of measuring the diameters of the zones of inhibition, measure the widths of the zones from any edge of the sample discs and the standard discs. The results obtained are multiplied by the factor 2 for determining whether the discs meet the requirements for uniformity prescribed by paragraph (f) of this section.

(f) The potency is satisfactory if the result obtained is not less than 67 percent and not more than 150 percent of that represented. The batch has a uniform potency if on the first or second test of six discs each, the diameter of the largest zone of inhibition is not more than 2.5 millimeters larger than the smallest zone, or if the number of zones that fall outside this range in three or more consecutive tests is not more than 10 percent of the total number of discs tested.

§ 460.15 Streptomycin sulfate discs for use in culture media.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Streptomycin sulfate discs for use in culture media are paper discs intended for impregnation of culture media in the sensitivity testing of mycobacteria. They conform to all requirements and to all procedures prescribed by § 460.1(a) for antibiotic sensitivity discs, except that each disc shall contain streptomycin sulfate equivalent to 10, 25, 50, or 500 micrograms of streptomycin.

(2) *Packaging.* It shall be packaged in accordance with the requirements of § 460.1(b).

(3) *Labeling.* In addition to complying with the requirements of § 460.1(c) of this chapter, the labeling shall also bear information indicating that the discs are for use in culture media for the sensitivity testing of mycobacteria and not for use in ordinary sensitivity disc plate tests.

(4) *Requests for certification; samples.* Requests for certification shall comply with § 460.1(d).

(b) *Tests and methods of assay; potency.* Proceed as directed in § 460.6 for the assay of streptomycin sulfate discs, except that:

(1) In the assay of streptomycin sulfate discs labeled to contain the equivalent of 10, 25, or 50 micrograms of streptomycin, the control discs shall be made to contain the equivalent of 6.25, 12.5, 25, 50, and 100 micrograms of streptomycin per disc.

(2) In the assay of streptomycin sulfate discs labeled to contain the equivalent of 500 micrograms of streptomycin:

(i) To each 100 milliliters of seed agar used for the test add 2.0 milliliters of suspension number 11.

(ii) The control discs shall be made to contain the equivalent of 50, 100, 200, 400, and 800 micrograms of streptomycin per disc.

Subpart B—Susceptibility Powders

§ 460.25 Bacitracin diagnostic sensitivity powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Bacitracin diagnostic sensitivity powder is bacitracin, with or without one or more suitable buffers and diluents, packaged in vials and intended for use in clinical laboratories for determining in vitro the sensitivity of microorganisms to bacitracin. Each vial contains 2,000 units of bacitracin. The potency of each immediate container is satisfactory if it contains not less than 90 percent and not more than 115 percent of its labeled content. It is sterile. Its loss on drying is not more than 5.0 percent. When reconstituted as directed in the labeling, its pH is not less than 5.5 and not more than 7.5. The bacitracin used conforms to the standards prescribed by § 448.10a(a) (1) (i), (v), and (vi) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. It shall be of appropriate size to permit the addition of 20 milliliters of sterile diluent when preparing a stock solution for use in making further dilutions for microbial susceptibility testing.

(3) *Labeling.* In addition to the requirements of § 432.5(a)(3) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container:

(*a*) The statement "For laboratory diagnostic use only."

(*b*) The statement "Sterile."

(*c*) The batch mark.

(*d*) The number of units of bacitracin in each immediate container.

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The bacitracin used in making the batch for potency, moisture, and pH.

(*b*) The batch for potency, sterility, loss on drying, and pH.

(ii) Samples required:

(*a*) The bacitracin used in making the batch: 6 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dilute an aliquot with 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), to the prescribed reference concentration.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

§ 460.28 Disodium carbenicillin diagnostic sensitivity powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Disodium carbenicillin diagnostic sensitivity powder is disodium carbenicillin packaged in vials and intended for use in clinical laboratories for determining in vitro the sensitivity of microorganisms to carbenicillin. Each vial contains disodium carbenicillin equivalent to not more than 1.0 gram of carbenicillin. The potency of each immediate container is satisfactory if it contains not less than 90 percent and not more than 120 percent of its labeled content. It is sterile. Its moisture content is not more than 6 percent. When reconstituted as directed in the labeling, its pH is not less than 6.0 and not more than 8.0. The disodium carbenicillin used conforms to the standards prescribed by § 440.13a(a)(1) (i), (v), (vi), and (vii) of this chapter.

(2) *Packaging.* It shall conform to the packaging requirements of § 432.1 of this chapter.

(3) *Labeling.* In addition to the requirements of § 432.5(a)(3) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container:

(*a*) The statement "For laboratory diagnostic use only."

(*b*) The statement "Sterile".

(*c*) The batch mark.

(*d*) The number of milligrams of carbenicillin in each immediate container.

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The disodium carbenicillin used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for potency, sterility, moisture, and pH.

(ii) Samples required:

(*a*) The disodium carbenicillin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dilute an aliquot with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 20 micrograms of carbenicillin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

§ 460.33 Clindamycin hydrochloride hydrate sensitivity powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Clindamycin hydrochloride hydrate diagnostic sensitivity powder is clindamycin hydrochloride hydrate packaged in vials and intended for use in clinical laboratories for determining in vitro the sensitivity of microorganisms to clindamycin. Each vial contains clindamycin hydrochloride hydrate equivalent to 20 milligrams of clindamycin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the amount of clindamycin it is represented to contain. It is sterile. Its moisture content is not more than 6.0 percent. Its pH in a solution containing 20 milligrams of clindamycin per milliliter is not less than 3.0 and not more than 6.5. The clindamycin hydrochloride hydrate used conforms to the standards prescribed by § 453.20(a)(1) (i), (ii), (iv), (v), (vi), and (vii) of this chapter.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. It shall be of appropriate size to permit the addition of 20 milliliters of sterile diluent in making further dilutions for microwhen preparing a stock solution for use bial susceptibility testing.

(3) *Labeling.* In addition to the requirements of § 432.5(a)(3) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container:

(*a*) The statement "For laboratory diagnosis only."

(*b*) The statement "Sterile".

(*c*) The batch mark.

(*d*) The number of milligrams of clindamycin in each immediate container.

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Request for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The clindamycin hydrochloride hydrate used in making the batch for clindamycin content, microbiological activity, moisture, pH, crystallinity, and identity.

(*b*) The batch for potency, sterility, moisture, and pH.

(ii) Samples required:

(*a*) The clindamycin hydrochloride hydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dilute an aliquot of this solution with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of clindamycin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the solution obtained when the vial is reconstituted with 1.0 milliliter of distilled water.

§ 460.38 Sodium colistimethate diagnostic sensitivity powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium colistimethate diagnostic sensitivity powder is sodium colistimethate packaged in vials and intended for use in clinical laboratories for determining in vitro the sensitivity of microorganisms to sodium colistimethate. Each vial contains sodium colistimethate equivalent to 100 milligrams of colistin base activity. It is sterile. Its moisture content is not more than 9.0 percent. When reconstituted as directed in the labeling, its pH is not less than 6.5 and not more than 9.0. It gives a positive identity test for sodium colistimethate. The sodium colistimethate used conforms to the standards prescribed by § 448.20a(a)(1) (i), (vi), (vii), and (viii) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. It shall be of appropriate size to permit the addition of 20 milliliters of sterile diluent when preparing a stock solution for use in making serial dilutions for microbial susceptibility testing.

(3) *Labeling.* Each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container.

(a) The statements "Not for therapeutic use" and "For laboratory diagnosis only".

(b) The statement "Sterile".

(c) The batch mark.

(d) The number of milligrams of colistin base activity in each immediate container.

(e) The statements "Stock solutions are stable for 14 days when refrigerated. For periods of storage up to 6 months, they should be frozen".

(f) Its expiration date which is 12 months, except that the date may be used that is 18, 24, 30, 36, 42, 48, 54, or 60 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that such drug as prepared by him is stable for such period of time. If the manufacturer or repacker of the drug has been exempted from the certification requirements, such date shall be the number of months after the month during which the batch was last assayed and released by the manufacturer or repacker.

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The sodium colistimethate used in making the batch for potency, moisture, pH, and identity.

(b) The batch for potency, sterility, moisture, pH, and identity.

(ii) Samples required:

(a) The sodium colistimethate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 30 immediate containers.

(2) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 448.20a(b)(1) of this chapter, except prepare the sample for assay as follows: Reconstitute as directed in the labeling and further dilute with 10 percent potassium phosphate buffer, pH 6.0, to the proper prescribed reference concentration. Its potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of colistin base activity that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) of this chapter.

(4) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using the drug reconstituted as directed in the labeling.

(5) *Identity.* Proceed as directed in § 448.20a(b)(7) of this chapter.

§ 460.42 Dihydrostreptomycin sulfate diagnostic sensitivity powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Dihydrostreptomycin sulfate sensitivity powder is crystalline dihydrostreptomycin sulfate, with or without one or more suitable buffers and diluents, packaged in vials and intended for use in clinical laboratories for determining in vitro the sensitivity of microorganisms to dihydrostreptomycin. Each vial contains dihydrostreptomycin sulfate equivalent to 20 milligrams of dihydrostreptomycin. The potency of each immediate container is satisfactory if it contains not less than 90 percent and not more than 115 percent of its labeled content. It is sterile. Its loss on drying is not more than 5.0 percent. When reconstituted as directed in the labeling, its pH is not less than 4.5 and not more than 7.0. The dihydrostreptomycin sulfate used conforms to the standards prescribed by § 444.10a(a)(1) of this chapter, except the standards for sterility, toxicity, pyrogens, and histamine. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. It shall be of appropriate size to permit the addition of 20 milliliters of sterile diluent when preparing a stock solution for use in making further dilutions for microbial susceptibility testing.

(3) *Labeling.* In addition to the requirements of § 432.5(a)(3) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container:

(a) The statement "For laboratory diagnostic use only".

(b) The statement "Sterile".

(c) The batch mark.

(d) The number of milligrams of dihydrostreptomycin in each immediate container.

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The dihydrostreptomycin sulfate used in making the batch for potency, moisture, pH, streptomycin content, and crystallinity.

(b) The batch for potency, sterility, loss on drying, and pH.

(ii) Samples required:

(a) The dihydrostreptomycin sulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 20 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dilute an aliquot with sterile distilled water to the prescribed reference concentration.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

§ 460.47 Doxycycline hyclate diagnostic sensitivity powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Doxycycline hyclate diagnostic sensitivity powder is crystalline doxycycline hyclate, with or without one or more suitable buffers and diluents, packaged in vials and intended for use in clinical laboratories for determining in vitro the sensitivity of microorganisms to doxycycline. Each vial contains doxycycline hyclate equivalent to 20 milligrams of doxycycline. The potency of

each immediate container is satisfactory if it contains not less than 90 percent and not more than 115 percent of its labeled content. It is sterile. Its moisture content is not more than 4 percent. When reconstituted as directed in the labeling, its pH is not less than 2.0 and not more than 3.5. The doxycycline hyclate used conforms to the standards prescribed by § 446.20(a)(1) (i), (iii), (iv), (v), and (vi) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. It shall be of appropriate size to permit the addition of 20 milliliters of sterile diluent when preparing a stock solution for use in making further dilutions for microbial susceptibility testing.

(3) *Labeling.* In addition to the requirements of § 432.5(a)(3) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container:

(a) The statement "For laboratory diagnostic use only".

(b) The statement "Sterile".

(c) The batch mark.

(d) The number of milligrams of doxycycline in each immediate container.

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The doxycycline hyclate used in making the batch for potency, moisture, pH, doxycycline content, identity, and crystallinity.

(b) The batch for potency, sterility, moisture, and pH.

(ii) Samples required:

(a) The doxycycline hyclate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 20 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Transfer a 10-milliliter aliquot to a 100-milliliter volumetric flask and dilute to volume with 0.1*N* hydrochloric acid. Further dilute an aliquot of this solution with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 0.1 microgram of doxycycline per milliliter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

§ 460.55 Lincomycin hydrochloride monohydrate diagnostic sensitivity powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Lincomycin hydrochloride monohydrate diagnostic sensitivity powder is lincomycin hydrochloride monohydrate powder packaged in vials and intended for use in clinical laboratories for determining in vitro the sensitivity of microorganisms to lincomycin. Each vial contains 20 milligrams of lincomycin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of lincomycin that it is represented to contain. It is sterile. Its moisture content is not more than 7 percent. It gives a positive identity test for lincomycin hydrochloride monohydrate. The lincomycin hydrochloride monohydrate used conforms to the standards prescribed by § 453.30(a)(1) (i), (iii), (iv), (v), and (ix) of this chapter.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass, and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. It shall be of appropriate size to permit the addition of 20 milliliters of sterile broth medium when preparing a stock solution for use in making serial dilutions for microbial susceptibility testing.

(3) *Labeling.* In addition to the requirements of §432.5(a)(3) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container:

(a) The statements "Not for therapeutic use" and "For laboratory diagnosis only".

(b) The statement "Sterile."

(c) The batch mark.

(d) The number of milligrams of lincomycin in each immediate container.

(e) The statements "Store in a refrigerator" and "Reconstituted solutions should be refrigerated."

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The lincomycin hydrochloride monohydrate used in making the batch for potency, moisture, pH, crystallinity, and specific rotation.

(b) The batch for potency, sterility, moisture, and identity.

(ii) Samples required:

(a) The lincomycin hydrochloride monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 30 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dilute an aliquot with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 2.0 micrograms of lincomycin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *Identity.* In a 100-milliliter beaker, dissolve sufficient sample to yield a concentration of at least 80 milligrams per milliliter, using no more than 2.0 milliliters of water. Add acetone until precipitation begins and then add an additional 20 milliliters of acetone. Filter the solution through filter paper and wash with two 10-milliliter portions of acetone. Expose the residue at room temperature until it is dry enough to be reduced to moderately fine particles. Dry the material for 4 hours in a 60° C. vacuum oven. After drying the material, care must be taken to avoid extended exposure to the atmosphere. The infrared spectrum of a mineral oil dispersion of the residue thus obtained exhibits maxima at the same wavelengths as that of the lincomycin working standard, similarly treated.

§ 460.58 Methacycline hydrochloride diagnostic sensitivity powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Methacycline hydrochloride diagnostic sensitivity powder is the crystalline methacycline hydrochloride, with or without one or more suitable buffers and diluents, packaged in vials and intended for use in clinical laboratories for determining in vitro the sensitivity of microorganisms to methacycline. Each vial contains methacycline hydrochloride equivalent to 20 milligrams of methacycline. The potency of each immediate container is satisfactory if it contains not less than 90 percent and not more than 115 percent of its labeled content. It is sterile. Its moisture content is not more than 4.0 percent. When reconstituted as directed in the labeling, its pH is not less than 2.0 and not more than 3.5. The methacycline hydrochloride used conforms to the standards prescribed by § 446.50(a)(1) (i), (iii), (v), and (vi) of this chapter.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container

as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. It shall be of appropriate size to permit the addition of 20 milliliters of sterile diluent when preparing a stock solution for use in making further dilutions for microbial susceptibility testing.

(3) *Labeling.* In addition to the requirements of § 432.5(a) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container:

(*a*) The statement "For laboratory diagnostic use only".

(*b*) The statement "Sterile".

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The methacycline hydrochloride used in making the batch for potency, moisture, absorptivity, identity, and crystallinity.

(*b*) The batch for potency, sterility, moisture, and pH.

(ii) Samples required:

(*a*) The methacycline hydrochloride used in making the batch: 10 packages, each containing 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample as follows: Reconstitute as directed in the labeling. Dilute an aliquot of this solution with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 0.06 microgram of methacycline per milliliter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

§ 460.64 Minocycline hydrochloride powder for microbial susceptibility testing.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Minocycline hydrochloride powder for microbial susceptibility testing is minocycline hydrochloride with or without one or more suitable buffers and diluents, packaged in vials and intended for use in clinical laboratories for determining in vitro the susceptibility of microorganisms to minocycline. Each vial contains minocycline hydrochloride equivalent to 20 milligrams of minocycline. The potency of each immediate container is satisfactory if it contains not less than 90 percent and not more than 115 percent of its labeled content. It is sterile. Its moisture content is not more than 5.0 percent. When reconstituted as directed in the labeling, its pH is not less than 2.0 and not more than 4.0. The minocycline hydrochloride used conforms to the standards prescribed by § 446.60(a)(1) (i), (iii), (iv), (v), (vi), and (vii) of this chapter.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass, and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. It shall be of appropriate size to permit the addition of 20 milliliters of sterile diluent when preparing a stock solution for use in making further dilutions for microbial susceptibility testing.

(3) *Labeling.* In addition to the requirements of § 432.5(a)(3) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container:

(*a*) The statement "For laboratory use only."

(*b*) The statement "Sterile."

(*c*) The batch mark.

(*d*) The number of milligrams of minocycline in each immediate container.

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The minocycline hydrochloride used in making the batch for potency, moisture, pH, minocycline content, identity, and crystallinity.

(*b*) The batch for potency, sterility, moisture, and pH.

(ii) Samples required:

(*a*) The minocycline hydrochloride used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dilute an aliquot with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 0.100 microgram of minocycline per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

§ 460.66 Oleandomycin phosphate diagnostic sensitivity powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oleandomycin phosphate diagnostic sensitivity powder is oleandomycin phosphate, with or without one or more suitable buffers and diluents, packaged in vials and intended for use in clinical laboratories, for determining in vitro the sensitivity of microorganisms to oleandomycin. Each vial contains oleandomycin phosphate equivalent to 20 milligrams of oleandomycin. The potency of each immediate container is satisfactory if it contains not less than 90 percent and not more than 115 percent of its labeled content. It is sterile. Its moisture content is not more than 5.0 percent. When reconstituted as directed in the labeling, its pH is not less than 4.0 and not more than 7.0. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. It shall be of appropriate size to permit the addition of 20 milliliters of sterile diluent when preparing a stock solution for use in making further dilutions for microbial susceptibility testing.

(3) *Labeling.* In addition to the requirements of § 432.5(a)(3) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container:

(*a*) The statement "For laboratory diagnostic use only."

(*b*) The statement "Sterile."

(*c*) The batch mark.

(*d*) The number of milligrams of oleandomycin in each immediate container.

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oleandomycin phosphate used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for potency, sterility, moisture, and pH.

(ii) Samples required:

(*a*) The oleandomycin phosphate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dilute an aliquot with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the prescribed reference concentration.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

§ 460.70 Oxytetracycline hydrochloride diagnostic sensitivity powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride diagnostic sensitivity powder is crystalline oxytetracycline hydrochloride, with or without one or more suitable buffers and diluents, packaged in vials and intended for use in clinical laboratories for determining in vitro the sensitivity of microorganisms to oxytetracycline. Each vial contains oxytetracycline hydrochloride equivalent to 20 milligrams of oxytetracycline. The potency of each immediate container is satisfactory if it contains not less than 90 percent and not more than 115 percent of its labeled content. It is sterile. Its loss on drying is not more than 2.0 percent. When reconstituted as directed in the labeling, its pH is not less than 2.0 and not more than 3.5. The oxytetracycline hydrochloride used conforms to the standards prescribed by § 446.67a(a)(1)(i), (vi), (vii), (viii), and (ix) of this chapter.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. It shall be of appropriate size to permit the addition of 20 milliliters of sterile diluent when preparing a stock solution for use in making further dilutions for microbial susceptibility testing.

(3) *Labeling.* In addition to the requirements of § 432.5(a)(3) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container:

(*a*) The statement "For laboratory diagnostic use only".

(*b*) The statement "Sterile".

(*c*) The batch mark.

(*d*) The number of milligrams of oxytetracycline in each immediate container.

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline hydrochloride used in making the batch for potency, moisture, pH, crystallinity, absorptivity, and identity.

(*b*) The batch for potency, sterility, loss on drying, and pH.

(ii) Samples required:

(*a*) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 30 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dilute an aliquot with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the prescribed reference concentration.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

§ 460.75 Potassium penicillin G diagnostic sensitivity powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Potassium penicillin G diagnostic sensitivity powder is crystalline potassium penicillin G, with or without one or more suitable buffers and diluents, packaged in vials and intended for use in clinical laboratories for determining in vitro the sensitivity of microorganisms to penicillin G. Each vial contains 20,000 units of penicillin G. The potency of each immediate container is satisfactory if it contains not less than 90 percent and not more than 115 percent of its labeled content. It is sterile. Its loss on drying is not more than 1.5 percent. When reconstituted as directed in the labeling, its pH is not less than 5.0 and not more than 7.5. The potassium penicillin G used conforms to the standards prescribed by § 440.80a(a)(1)(i), (v), and (vi) of this chapter.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. It shall be of appropriate size to permit the addition of 20 milliliters of sterile diluent when preparing a stock solution for use in making further dilutions for microbial susceptibility testing.

(3) *Labeling.* In addition to the requirements of § 432.5(a)(3) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container:

(*a*) The statement "For laboratory diagnostic use only".

(*b*) The statement "Sterile".

(*c*) The batch mark.

(*d*) The number of units of penicillin G in each immediate container.

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The penicillin G used in making the batch for potency, moisture, pH, and crystallinity.

(*b*) The batch for potency, sterility, loss on drying, and pH.

(ii) Samples required:

(*a*) The potassium penicillin G used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) Potency. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dilute an aliquot with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the prescribed reference concentration.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) or (2) of that section, except if using the method in paragraph (e)(2), use medium B in lieu of medium A.

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

§ 460.79 Polymyxin B sulfate diagnostic sensitivity powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Polymyxin B sulfate diagnostic sensitivity powder is polymyxin B sulfate, with or without one or more suitable buffers and diluents, packaged in vials and intended for use in clinical laboratories for determining in vitro the sensitivity of microorganisms to polymyxin B. Each vial contains the equivalent of 20,000 units of polymyxin B. The potency of each immediate container is satisfactory if it contains not less than 90 percent and not more than 115 percent of its labeled content. It is sterile. Its loss on drying is not more than 7.0 percent. When reconstituted as directed in the labeling, its pH is not less than 5.0 and not more than 7.5. The polymyxin B sulfate used conforms to the standards prescribed by § 448.30a(a)(1)(i), (v), (vi), and (ix) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. It shall be of appropriate size to permit the addition of 20 milliliters of sterile diluent when preparing a stock solution for use in making further dilutions for microbial susceptibility testing.

(3) *Labeling.* In addition to the requirements of § 432.5(a)(3) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container:

(*a*) The statement "For laboratory diagnostic use only".

(*b*) The statement "Sterile".

(*c*) The batch mark.

(*d*) The number of units of polymyxin B in each immediate container.

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The polymyxin B sulfate used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for potency, sterility, loss on drying, and pH.

(ii) Samples required:

(*a*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dilute an aliquot with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the prescribed reference concentration.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

§ 460.86 Spectinomycin hydrochloride powder for microbial susceptibility testing.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Spectinomycin hydrochloride powder for microbial susceptibility testing is spectinomycin dihydrochloride pentahydrate with or without one or more suitable buffers and diluents, packaged in vials and intended for use in clinical laboratories for determining in vitro the susceptibility of microorganisms to spectinomycin. Each vial contains spectinomycin hydrochloride equivalent to 100 milligrams of spectinomycin. The potency of each immediate container is satisfactory if it contains not less than 90 percent and not more than 115 percent of its labeled content. It is sterile. Its moisture content is not more than 8 percent. When reconstituted as directed in the labeling, its pH is not less than 3.8 nor more than 5.6. The spectinomycin hydrochloride used conforms to the standards prescribed by § 455.80a(a)(1) (i), (ii), (vii), (viii), (ix), (x), and (xi) of this chapter.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. It shall be of appropriate size to permit the additon of the amount of sterile diluent prescribed in the labeling when preparing a stock solution for use in making further dilutions for microbial susceptibility testing.

(3) *Labeling.* In addition to the requirements of § 432.5(a)(3) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container:

(*a*) The statement, "For laboratory use only".

(*b*) The statement, "Sterile".

(*c*) The batch mark.

(*d*) The number of milligrams of spectinomycin in each immediate container.

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The spectinomycin hydrochloride used in making the batch for spectinomycin content, microbiological activity, moisture, pH, identity, residue on ignition, and crystallinity.

(*b*) The batch for potency, sterility, moisture, and pH.

(ii) Samples required:

(*a*) The spectinomycin hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dilute an aliquot with sterile distilled water to the prescribed reference concentration.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

§ 460.89 Streptomycin sulfate diagnostic sensitivity powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Streptomycin sulfate diagnostic sensitivity powder is streptomycin sulfate, with or without one or more suitable buffers and diluents, packaged in vials and intended for use in clinical laboratories for determining in vitro the sensitivity of microorganisms to streptomycin. Each vial contains streptomycin sulfate equivalent to 20 milligrams of streptomycin. The potency of each immediate container is satisfactory if it contains not less than 90 percent and not more than 115 percent of its labeled content. It is sterile. Its loss on drying is not more than 5.0 percent. When reconstituted as directed in the labeling, its pH is not less than 4.5 and not more than 7.0. The streptomycin sulfate used conforms to the standards prescribed by § 444.70a(a)(1) (i), (vi), and (vii) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. It shall be of appropriate size to permit the addition of 20 milliliters of sterile diluent when preparing a stock solution for use in making further dilutions for microbial susceptibility testing.

(3) *Labeling.* In addition to the requirements of § 432.5(a)(3) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container:

(*a*) The statement "For laboratory diagnostic use only".

(*b*) The statement "Sterile".

(*c*) The batch mark.

(*d*) The number of milligrams of streptomycin in each immediate container.

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The streptomycin sulfate used in making the batch for potency, loss on drying, and pH.

(*b*) The batch for potency, sterility, loss on drying, and pH.

(ii) Samples required:

(*a*) The streptomycin sulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 20 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dilute an aliquot with sterile distilled water to the prescribed reference concentration.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

§ 460.93 Tetracycline hydrochloride diagnostic sensitivity powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline hydrochloride diagnostic sensitivity powder is crystalline tetracycline hydrochloride, with or without one or more suitable buffers and diluents, packaged in vials and intended for use in clinical laboratories for determining in vitro the sensitivity of microorganisms to tetracycline. Each vial contains 20 milligrams of tetracycline hydrochloride. The potency of each immediate container is satisfactory if it contains not less than 90 percent and not more than 115 percent of its labeled content. It is sterile. Its loss on drying is not more than 2.0 percent. When reconstituted as directed in the labeling, its pH is not less than 1.8 and not more than 3.0. The tetracycline hydrochloride used conforms to the standards prescribed by § 446.81a(a)(1) (i), (vi), and (vii), and (viii) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. It shall be of appropriate size to permit the addition of 20 milliliters of sterile diluent when preparing a stock solution for use in making further dilutions for microbial susceptibility testing.

(3) *Labeling.* In addition to the requirements of § 432.5(a)(3) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container:

(a) The statement "For laboratory diagnostic use only."

(b) The statement "Sterile."

(c) The batch mark.

(d) The number of milligrams of tetracycline hydrochloride in each immediate container.

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The tetracycline hydrochloride used in making the batch for potency, moisture, pH, crystallinity, and absorptivity.

(b) The batch for potency, sterility, loss on drying, and pH.

(ii) Samples required:

(a) The tetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 500 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 20 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dilute an aliquot with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the prescribed reference concentration.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter using the drug reconstituted as directed in the labeling.

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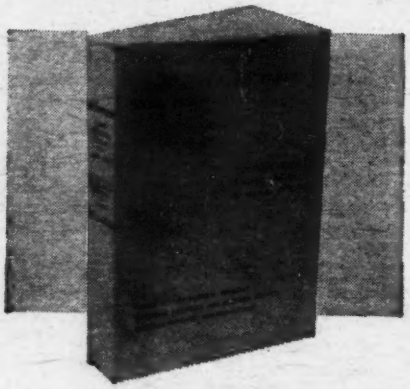
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